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SOME NUTRITIONAL DISORDERS OF THE TOMATO

BY W. F. BEWLEY, D.Sc.
AND H. L. WHITE, B.A., DIP. HORT. (CANTAB.)
(*Experimental and Research Station, Cheshunt, Herts.*)

(With Plate VI and 1 Text-figure.)

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I. BLOTCHY RIPENING OF TOMATO FRUITS.

IRREGULAR ripening spoils the quality and appearance of tomato fruits, and no matter how perfect their size and shape may be, they must be packed as "roughs" if they do not ripen to a uniformily red colour. In many cases the percentage of unsightly fruit is as low as 0·5 or 1 per cent., but under unfavourable conditions it may rise as high as 50 per cent., thus seriously lowering the market value of the crop.

(1) *The different kinds of irregularly ripened fruits.*

Irregularly ripened tomato fruits in this country may be divided into four classes, illustrated in Plate VI.

(*a*) Fruits suffering from "Stripe" disease caused by *Bacillus lathyri*. The surface of infected fruits shows sunken, brown irregular pits and markings.

(*b*) Fruits suffering from Aucuba Mosaic disease, due to a filterable virus. The various markings have sharply defined edges. The fruits may be completely white, or may show flecks of brilliant red or orange scattered over a silvery white background. Usually, patches of normal red, alternate with areas of green, yellow and orange, sometimes in a manner reminiscent of a "Paisley" shawl. Normally the contour of the fruit is smooth, but in bad cases the surface may be irregular and covered with pimples.

(*c*) Fruits suffering from "green back" or "hard back." These fruits possess a hard green area round the stalk-end, while the rest of the fruit softens and turns red. As the ripening processes continue, the affected area may either remain green or become orange in colour, but invariably it remains hard, even though the rest of the fruit becomes over-ripe. In section the flesh immediately below the hard green area is opaque, hard and green, whereas the other tissues are clear, soft and red. The vascular bundles within the hard flesh are usually green in colour, and necrosis of the bundles has not been observed. The cells of the hard flesh are in an immature condition, are small and have not swollen. The flesh is less mucilaginous, and frequently the wall of the fruit is thinner than in the riper areas.

(*d*) *True blotchy ripening.* In fruits affected by this disorder the hard green areas are not confined to the stalk end, but may arise in any position. Usually there is no abrupt line of demarcation between the green and red areas: the colours merge gradually. The observations previously recorded concerning the hard areas in "green back" apply equally well in the case of "blotchy ripening," but in the latter case the vascular bundles are sometimes brown and necrotic. In the most severe cases, the hard areas are not opaque and green, but are waxy or glassy in appearance, and thus give rise to the term "waxy patch" used by nurserymen. The bundles lying beneath these clear, glassy areas are invariably brown and necrotic, a fact that may be easily observed from the outside. An attempt has been made to correlate the position of the affected areas with that of the various parts of the vascular system of

the fruit cortex. These areas usually arise along the furrow immediately above a wall of one of the carpels composing the fruit. Occasionally they have been found immediately above the main bundle of the carpel.

An attempt to dissect healthy fruits, so as to expose the vascular system proved of little value, owing to the delicate nature of the individual strands, and "clearing" methods were adopted. By carefully removing the skin of a half ripe tomato, and scraping away the superficial tissue, the external portion of the vascular system can be exposed, as shown in Text-fig. 1, fig. 1. It will be noticed that the veins radiate from the stalk, and that in the top half of the fruit there are few cross-connections, while in the bottom half the veins anastomose to form an intricate network. By clearing the prepared fruit in xylol following alcohol, a good idea of the vascular system of the entire fruit can be obtained.

Examination of blotchy fruits in the above manner, revealed certain differences between their bundles and those of healthy fruits. The bundles of blotchy fruits seem somewhat thicker than those of healthy fruits, owing to the spongy nature of cells immediately surrounding them. Necrosis of the bundles frequently occurs. Text-fig. 1, fig. 2 shows the veins at the lower side of a blotchy fruit radiating from the centre, which originally held the style of the flower. Necrosis of the bundles can be seen at the points marked *A*, which were immediately beneath distinct blotches. Text-fig. 1, fig. 3, shows an affected bundle with an adjacent group of corky cells (cross shaded). Such corky cells occur frequently in blotchy fruits.

A section through a necrotic bundle, viewed by means of a small hand lens, almost invariably shows the presence of gaps or canals in the parenchyma bordering the bundle (see Text-fig. 1, fig. 4). The necrotic bundle *B* is bordered on two sides by canals marked *A*. The walls of these canals are usually brown and necrotic.

(2) *The incidence of blotchy ripening.*

(*a*) *Seasonal distribution.* The symptoms, as in many nutritional disorders, are most severe about the months of June and July. Fruit ripening in September and October may be completely free from blotchy ripening, although the same plants in the early part of the season—late May, June and early July—may have borne fruit, 45 per cent. of which was blotchy. Table I gives the percentage of blotchy fruits picked throughout the season, during five years, from the plot receiving nitrogenous and phosphatic fertilisers but no potash.

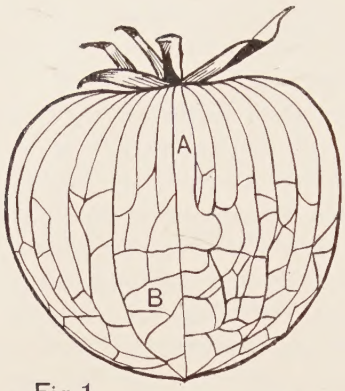

Fig.1

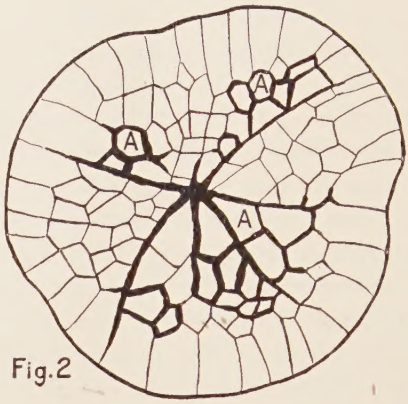

Fig.2

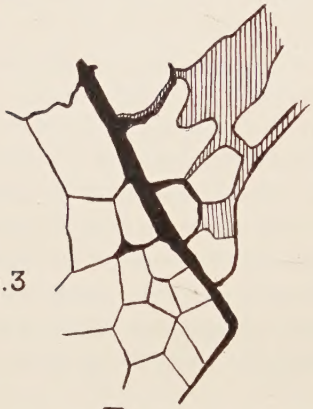

Fig.3

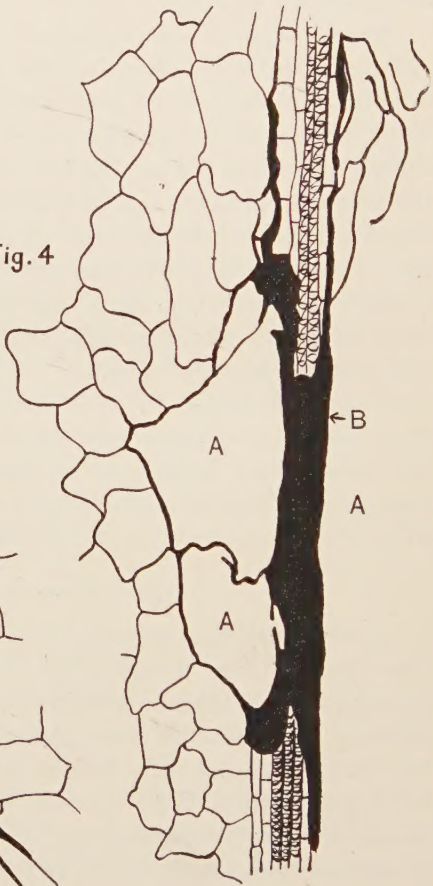

Fig.4

Fig.5

Text-fig. 1.

Table I.

Seasonal distribution of blotchy fruit.

Month	Percentage of blotchy fruits*				
	1921	1922	1923	1924	1925
June	28	19	11	18	32
July	45	36	33	14	27
August	8	22	12	4	37
September	2	43	10	5	3
October	—	—	15	10	—

* In 1921 and 1922 the variety grown was Kondine Red and in 1923, 1924 and 1925 Manx Marvel.

(*b*) *Varietal susceptibility.* A certain amount of varietal susceptibility to the causes underlying blotchy ripening undoubtedly exists. No variety remains consistently free from this disorder, but Kondine Red is so susceptible that this fact alone may cause its elimination when selecting varieties for commercial use. The variety Comet produces good quality fruit much less susceptible to imperfect ripening than that of Kondine Red. Thus the average percentage of blotchy fruit from Comet on an unmanured plot during 1921 and 1922 was 27·9, whereas Kondine Red under similar conditions gave 46·9 per cent. of this type of fruit.

(3) *The effect of manurial treatment on blotchy ripening.*

(*a*) *The relation of blotchy ripening to general starvation.* The frequent association of this disorder with unhealthy and starved-looking plants suggests that it is the result of malnutrition, and during the past five years a considerable amount of evidence in favour of this assumption has been obtained at the Cheshunt Station.

In 1923 a block of four houses was erected on grass land which had been undisturbed for many years. These houses were set apart for experiments, which necessitated leaving one house unmanured, while the remaining three were given liberal dressings and every effort made to obtain a good crop. The crop yields for the first year as given in Table II clearly show that the percentage of blotchy fruit averaging 4·15 per cent. in the well-manured houses and 49·75 per cent. in the unmanured

Table II.

Percentage of blotchy fruit from the plots in houses 7 *to* 10 *during* 1923.

House 7 manured		House 8 manured		House 9 manured		House 10 unmanured	
Fallow	Fallow	9·43	7·40	6·65	5·79	Fallow	Fallow
5·45	5·89	12·58	8·32	5·15	5·78	48·55	49·01
4·66	4·71	5·32	7·92	6·99	8·95	53·91	50·66
2·30	1·90	5·42	4·58	3·24	4·10	45·14	43·94

house is dependent upon manurial treatment. Similar results were obtained in 1924 and 1925.

(*b*) *The relation of blotchy ripening to nitrogenous, phosphatic, and potassic nutrition.* Examination of the fruits collected from the various experimental houses at Cheshunt has yielded information concerning the influence of different fertilisers on the ripening of the fruits. Two houses, which have been particularly useful, are 27 ft. wide and 100 ft. long. They were erected in 1915 and since 1916 each has been divided into eight equal plots 25 ft. long by 12 ft. wide containing 120 plants and occupying $\frac{1}{145}$th of a house-acre. From 1916 to 1920 the results from these plots were measured as total weight of fruit collected. From 1921 to 1925 the fruit collected was graded and weighed. In this way the effect of any particular treatment upon quality as well as quantity may

Table III.

Relation of blotchy fruit to manurial treatment.

Treatment	Percentage of blotchy fruit					Average percentage of blotchy fruit during the 5 years 1921–1925	Average crop in tons per acre during the 5 years 1921–1925
	Kondine Red		Manx Marvel				
	1921	1922	1923	1924	1925		
K 1 Double complete artificials	1·97	0·99	1·85	0·62	1·40	1·31	36·02
K 2 Unmanured	57·16	36·65	18·45	24·45	43·25	35·93	29·24
K 3 Standard treatment + complete artificials	2·77	1·90	2·28	0·38	1·95	1·86	38·24
K 4 Complete artificials + dung	4·79	0·92	2·01	0·21	0·41	1·69	39·69
K 5 Complete artificials	5·83	0·37	2·59	0	0	2·93	41·40
K 6 Complete artificials less phosphates	6·37	0·21	2·52	0·61	1·35	2·21	37·46
K 7 Complete artificials less nitrogen	23·99	7·15	8·73	2·07	8·92	10·17	35·45
K 8 Complete artificials less potash	24·23	20·15	18·12	12·21	24·87	19·92	36·71
	Comet		Manx Marvel				
J 1 Double complete artificials	3·11	0	2·22	2·43	0·62	1·68	36·05
J 2*a* Unmanured	47·18	8·64	25·17	51·05	33·23	33·05	30·84
J 2*b* Stable manure only	—	—	—	—	1·90	1·90	35·80
J 5 Complete artificials	12·73	0	2·16	0·32	0·94	3·23	38·14
J 6 Complete artificials less phosphates	8·75	0	1·60	2·22	0·71	2·66	37·42
J 7 Complete artificials less nitrogen	27·65	0·28	10·23	3·75	0·60	9·70	33·51
J 8 Complete artificials less potash	31·83	1·52	22·39	18·07	23·04	19·37	35·22

be ascertained. One grade, separated in this way, is the grade of blotchy fruits. In Table III is shown the percentage of blotchy fruits obtained from the various plots in two similar houses. These plots received the same treatment each year since 1916, except plots 1, 2 and 3 in both houses which were started in 1919.

The outstanding feature of the results is the high proportion of blotchy fruits occurring on the unmanured plot, and those plots from which potash and nitrogen respectively are withheld. On the remaining plots blotchy fruit is not absent but it is negligible in quantity and rarely rises above 3 per cent. The greatest proportion of blotchy fruits occurs on the unmanured plots, averaging over a period of five years 33 per cent. in one house and 35·9 per cent. in the other.

The average of the affected fruits over the same period from the potash-starved plot is 19·3 per cent. in the first and 19·9 per cent. in the second house, while the average from the nitrogen-starved plot is 6·6 per cent. and 8·9 per cent. respectively. The fruits from the phosphate-starved plots are only slightly affected, the five years' average being 0·7 per cent. in one house and 1·3 per cent. in the other. The proportion of blotchy fruits from the liberally manured plots is noticeably low in both houses. It is to be noted that the proportion of blotchy fruits bears no relation to the size of the crop. Thus the plot receiving a double dressing of complete artificials has yielded a lower average crop than that receiving a single dressing, while the proportion of blotchy fruits from the former has been uniformly less than that from the latter.

(*c*) *The elimination of blotchy fruits by addition of potassic and nitrogenous fertilisers.* The results of the previous experiments suggest that the addition of potassic and nitrogenous fertilisers to soil starved in respect of these nutrients will cause a corresponding reduction in the proportion of blotchy fruits, and experiments were arranged accordingly.

In 1923, three plots starved in phosphates, potash and nitrogen respectively were each subdivided into four equal sections to each of which was added different quantities of the particular fertiliser in which the plot was deficient. Thus the plot without phosphates was divided into four equal plots, one of which was without phosphate as before, the second had a mixture containing 8 per cent. phosphates, the third 16 per cent. and the fourth 24 per cent. The nitrogen-starved plot was divided into four plots; one was left without nitrogen, the others had $1\frac{1}{4}$ per cent., $2\frac{1}{2}$ per cent. and 5 per cent. nitrogen respectively. The plot deficient in potash was also divided into four plots: one without, the others with $2\frac{1}{2}$ per cent., 5 per cent. and 7 per cent. potash respectively. Nitrogen was added as sulphate of ammonia, potash as sulphate of

potash and phosphate as superphosphate. These treatments were repeated in 1924, and in 1925 the amounts of the different nutrients were uniformly increased as shown in Table IV. The results given in Table IV show that the percentage of blotchy fruits is reduced by the application of nitrogen as sulphate of ammonia and potash as sulphate of potash. Increased dressings of phosphates as superphosphate have not materially affected the proportion of these fruits, and it is clear that phosphate is not a factor involved in their production.

Table IV.

Elimination of blotchy fruit on starved plots by addition of potassic and nitrogenous fertilisers.*

Percentage of phosphates in fertiliser	0	8	16	24	32
Percentage of blotchy fruit 1923	1·60	0·68	2·61	2·98	—
,, ,, ,, 1924	2·22	1·37	0·18	0·44	—
,, ,, ,, 1925	0·71	—	0·36	0·19	0·19
Average	1·51	1·03	1·05	1·20	0·19
Percentage of nitrogen in fertiliser	0	1¼	2½	5	7½
Percentage of blotchy fruit 1923	10·23	4·73	3·98	2·00	—
,, ,, ,, 1924	3·75	0·78	0·16	0·27	—
,, ,, ,, 1925	6·60	—	0·66	0·96	0·70
Average	6·86	2·75	1·60	1·08	0·70
Percentage of potash in fertiliser	0	2½	5	7½	10
Percentage of blotchy fruit 1923	22·39	12·30	12·16	14·80	—
,, ,, ,, 1924	18·07	6·22	4·39	3·57	—
,, ,, ,, 1925	25·04	—	4·08	2·96	0·91
Average	21·83	9·26	6·88	7·11	0·91

* The forms of nitrogen, phosphate and potash used in this experiment were sulphate of ammonia, superphosphate and sulphate of potash respectively.

(*d*) *The effect of stable manure on blotchy ripening.* In 1925, a plot unmanured for six years was divided into two equal sections; one was left unmanured as before, while stable manure at the rate of 14 lb. per sq. yd. was dug into the soil of the other section 28 days before planting.

The resulting crop showed 33·05 per cent. of blotchy fruit in the unmanured section and 1·9 per cent. in the manured section.

(4) *The effect of climatic factors on blotchy ripening.*

It is apparent from Table III that a certain proportion of the fruits become blotchy even when the soil is liberally manured. For example, the amount of blotchy fruits for a period of five years on the plots receiving complete artificials has been as follows:

	1st house	2nd house
1921	12·73	5·83
1922	0	0·37
1923	2·16	2·59
1924	0·32	0
1925	0·94	0

Plainly some other factor or factors influence the minimum amount of blotchy ripening, the annual variation of which is approximately similar in the two houses. Climatic factors at once suggest themselves as the cause of this variation and while further study is necessary before reliable conclusions may be drawn there are indications that sunlight is one important factor.

Table III suggests that the effect of sunlight on the ripening of tomato fruits coincides to some extent with that of potassic fertilisers, for the proportion of blotchy fruits from the nitrogen-starved plots in 1921, which was a sunny year, approximates to that from the potash-starved plots. In the less sunny years, 1922 and 1923 there were considerably more blotchy fruits from the potash-starved plots than from the nitrogen-starved plots. Also during the very dull season of 1924, the ratio was still further increased.

(5) *The effect of root disease on blotchy ripening.*

Cases have been observed of single plants in well-manured plots producing an abnormal amount of blotchy fruits. The plants have invariably been weak specimens and examination of the roots showed them to be badly attacked by the root fungus, *Colletotrichum tabificum*.

(6) *Discussion of results.*

The foregoing experimental results leave no doubt that blotchy ripening of tomato fruits is connected with an insufficient supply of available potassic and nitrogenous foods; phosphate deficiency appears to be unimportant. Further, the feeding of affected plants with potassic and nitrogenous fertilisers causes a corresponding reduction in the quantity of fruits affected. Under the most favourable manurial conditions, however, a small quantity of blotchy fruits is invariably produced, and it is apparent that other factors are operative in a small way. The remarkable response to potash deficiency is not altogether surprising because analysis of tomato fruits has shown them to be very rich in potash. Thus Patterson[1] who made 612 determinations found an average of 67·3 per cent. of potash in the ash of tomato fruits.

[1] Patterson, J., Report of the Chemist, *Md. Agr. Exp. Sta. Ann. Report*, 1899.

Although potash and nitrogen are both important factors in the ripening of tomato fruits, the former is by far the more important of the two. In actual advisory work it has been found that the application of sulphate of potash at the rate of 5 cwts. per acre is sufficient to correct blotchy ripening on average soils. On the other hand, cases are known where the soil is so unbalanced that three tons sulphate of potash per acre had to be applied before blotchy ripening was reduced to negligible proportions. In cases where both potash and nitrogen are deficient, two dressings at an interval of ten days each of a mixture of $2\frac{1}{2}$ cwts. sulphate of ammonia and $2\frac{1}{2}$ cwts. sulphate of potash per acre has proved successful.

It should be borne in mind, however, that all plants grown in soils liberally provided with potash and nitrogen are not necessarily well nourished in respect to these nutrients. The nourishment of the plant depends upon a healthy root action and sufficient water in the soil to carry the nutrients. Realisation of these simple facts has frequently solved growers' problems, which soil analysis has failed to elucidate. Thus tomato plant roots are frequently parasitised by a fungus *Colletotrichum tabificum* which appreciably reduces the size of the functioning root system. Plants affected in this way cannot be well nourished, although growing in fertile soil.

Again a common fault in tomato cultivation is dryness of the soil below the top six inches. If this section of the soil is once allowed to become dry, it is exceedingly difficult to wet it during the growing season, and the result is detrimental to the plant. In several cases blotchy ripening has been traced to a dry base, and corrected by taking special measures to wet it.

The position regarding "green back" is not so simple. Susceptibility to this disorder bears in general the same relation to environmental factors as blotchy ripening, being increased by a deficiency of potash and nitrogen, but the ultimate symptoms seem to be intensified by exposure of the fruits to strong sunlight.

Duggar[1] showed that when tomato fruits, which are normally red when ripe, are ripened at 90° F. or above, they become yellow instead of red when fully ripe. He further showed that tomatoes ripened in the absence of oxygen also became yellow instead of red. Thus the red pigment, lycopin, does not develop at high temperatures and in the absence of oxygen. In "green back" the affected tissues do not develop lycopin but assume an orange-yellow colour.

[1] Duggar, B. M. (1913), *Washington University Studies*, vol. I, pt. 1, No. 1

It is reasonable to assume that the paucity of this pigment is due to the heating of the tissues by exposure to the direct rays of the sun. This assumption is confirmed by observation of plants grown in commercial nurseries, for the fruits from plants with scanty foliage, or those trimmed too severely, have almost invariably shown a high proportion of "green back." The prevention of "green back" seems to depend upon liberal manuring with potassic fertilisers and suitable shading of the glass during the height of the summer.

II. Leaf Scorch.

Striking differences have been observed in the appearance of the foliage of tomato plants grown in the plots from which potash and nitrogen respectively have been omitted for the last ten years.

The experiments were commenced in 1916 and foliage differences became apparent in 1920.

(1) *Foliage of plants grown in soil manured with phosphates and potash while nitrogen is withheld.*

The foliage is generally paler in colour than normal foliage and the leaf area is much reduced. Towards the middle of June, when the crop borne by the five lower trusses is exercising a severe strain on the foliage, pale yellow areas appear between the veins. The yellowing develops towards the edge of the leaves until the entire surface is affected.

(2) *Foliage of plants grown in soil manured with phosphates and nitrogen while potash is withheld.*

The colour of the foliage is abnormally dark green and frequently purplish brown in colour. Towards the middle of June, a paling of the margin of the leaf becomes evident. The pale coloration gradually spreads inwards between the veins and is followed by browning of the tissues as the margins of the leaflets dry out. Finally the whole leaf becomes brown and desiccated. Side shoots which develop towards the end of the season are usually dark green in colour showing no signs of scorch in the early stages of their development: later they may develop scorch. Quite frequently, however, the young shoots develop scorch at an early stage.

The scorching of the foliage appears each year in the potash-starved plots. The subdivision of these plots in 1923, and the consequent addition of increasing quantities of sulphate of potash to the sub-plots,

Table V.

Sand culture experiments during 1924.

Series No.	Treatment	Set	Fertiliser in ounces per 12-inch pot: Sulphate of ammonia	Fertiliser in ounces per 12-inch pot: Superphosphate	Fertiliser in ounces per 12-inch pot: Sulphate of potash	Foliage symptoms	Remarks
I.	Untreated	1	.	.	.	Leaflets stunted and light in colour but show no scorch	
II.	Increasing amounts of sulphate of ammonia in the absence of phosphate and potash	2	¼	.	.	Leaflets with slight yellowing of the margins surrounding an abnormally dark green centre	The addition of ¼ oz. sulphate of ammonia in the absence of potash and phosphate causes marginal yellowing, which increases in intensity as the dressing of sulphate of ammonia is increased
		3	½	.	.	Leaflets with more extensive marginal yellowing	
		4	¾	.	.	Edges of leaves curled back and drying out	
III.	Increasing amounts of sulphate of ammonia in the presence of superphosphate	5	¼	¼	.	Yellowing of the margins as in set 3	The addition of superphosphate in the presence of increasing amounts of sulphate of ammonia increases the intensity of the symptoms
		6	½	¼	.	Typical marginal leaf-scorch as in set 4. Plants more stunted than in set 3	
		7	¾	¼	.	Very severe leaf-scorch. Plants more stunted than in set 4	
IV.	Increasing amounts of sulphate of ammonia in the presence of superphosphate and sulphate of potash	11	¼	¼	¼	Foliage healthy. No scorch	The addition of potash completely removes all symptoms of scorch
		12	½	¼	¼	Foliage healthy. No scorch	
		13	¾	¼	¼	Foliage healthy. No scorch	

V.	Increasing amounts of superphosphate in the absence of potash and nitrogen	14	.	$\frac{1}{4}$	.	Leaflets pale in colour and very starved-looking but no scorch	Plants without potash, *but also without nitrogen* show no scorch
		15	.	$\frac{1}{2}$	.	Leaflets pale in colour and very starved-looking but no scorch	
		16	.	$\frac{3}{4}$	.	Leaflets pale in colour and very starved-looking but no scorch	
VI.	Increasing amounts of phosphate in the presence of nitrogen and the absence of potash	20	$\frac{1}{4}$	$\frac{1}{4}$	.	Leaflets with yellowing of the margins and in places typical marginal scorch	A similar series without potash *but with the addition of nitrogen* shows typical scorch
		21	$\frac{1}{4}$	$\frac{1}{2}$	.	Leaflets with yellowing of the margins and in places typical marginal scorch	
		22	$\frac{1}{4}$	$\frac{3}{4}$	.	Leaflets with yellowing of the margins and in places typical marginal scorch	
VII	Increasing amounts of phosphate in the presence of both potash and nitrogen	23	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	Foliage healthy. No scorch	The addition of potash again ensures freedom from symptoms of scorch
		24	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{4}$	Foliage healthy. No scorch	
		25	$\frac{1}{4}$	$\frac{3}{4}$	$\frac{1}{4}$	Foliage healthy. No scorch	
VIII	Increasing amounts of sulphate of potash only	26	.	.	$\frac{1}{4}$	Reduction of total leaf-area compared with series IV, VII and X, but foliage normal in colour and no scorch	In this and series IX and X potash is never deficient and there is no signs of scorch
		27	.	.	$\frac{1}{2}$	Reduction of total leaf-area compared with series IV, VII and X, but foliage normal in colour and no scorch	
		28	.	.	$\frac{3}{4}$	Reduction of total leaf-area compared with series IV, VII and X, but foliage normal in colour and no scorch	
IX	Increasing amounts of sulphate of potash in the presence of nitrogen and the absence of phosphate	29	$\frac{1}{4}$	.	$\frac{1}{4}$	Foliage healthy. No scorch	
		30	$\frac{1}{4}$	.	$\frac{1}{2}$	Foliage healthy. No scorch	
		31	$\frac{1}{4}$	.	$\frac{3}{4}$	Foliage healthy. No scorch	
X	Increasing amounts of sulphate of potash in the presence of nitrogen and phosphate	35	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	Strong healthy plants. No scorch	
		36	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{2}$	Strong healthy plants. No scorch	
		37	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{3}{4}$	Strong healthy plants. No scorch	

Table VI.

Sand culture experiments during 1925.

Set		Fertiliser in ounces per 12-inch pot: Ammonium sulphate	Super-phosphate	Potassium sulphate	Symptoms*
I	1	.	.	.	−
	2	1/32	.	.	−
	3	1/16	.	.	−
	4	1/8	.	.	−
	5	1/4	.	.	−
	6	1/2	.	.	+
II	7	1/32	1/8	.	−
	8	1/16	1/8	.	+
	9	1/8	1/8	.	+
	10	1/4	1/8	.	+ +
	11	1/2	1/8	.	+ + +
III	12	1/32	1/8	1/8	−
	13	1/16	1/8	1/8	−
	14	1/8	1/8	1/8	−
	15	1/4	1/8	1/8	−
	16	1/2	1/8	1/8	−
IV	17	.	1/32	.	−
	18	.	1/16	.	−
	19	.	1/8	.	−
	20	.	1/4	.	−
	21	.	1/2	.	−
V	22	1/8	1/32	.	−
	23	1/8	1/16	.	−
	24	1/8	1/8	.	+
	25	1/8	1/4	.	+ +
	26	1/8	1/2	.	+ +

Set		Fertiliser in ounces per 12-inch pot: Ammonium sulphate	Super-phosphate	Potassium sulphate	Symptoms
VI	27	1/8	1/32	1/8	−
	28	1/8	1/16	1/8	−
	29	1/8	1/8	1/8	−
	30	1/8	1/4	1/8	−
	31	1/8	1/2	1/8	−
VII	32	.	.	1/32	−
	33	.	.	1/16	−
	34	.	.	1/8	−
	35	.	.	1/4	−
	36	.	.	1/2	−
VIII	37	1/8	.	1/32	−
	38	1/8	.	1/16	−
	39	1/8	.	1/8	−
	40	1/8	.	1/4	−
	41	1/8	.	1/2	−
IX	42	1/8	1/8	1/32	−
	43	1/8	1/8	1/16	−
	44	1/8	1/8	1/8	−
	45	1/8	1/8	1/4	−
	46	1/8	1/8	1/2	−

* − = no scorch. + = marginal scorch. + + = moderate scorch. + + + = bad scorch.

Table VII.

The effect of increasing amounts of superphosphate in the presence of ammonium sulphate on the intensity of leaf scorch of the tomato.

Series	Treatment in ounces per 12-inch pot: Ammonium sulphate	Super-phosphate	Potassium sulphate	Number of leaves: Unscorched	Scorched	Number of scorched leaves in comparison with unscorched reckoned as 10
26 Pot 1	1/8	1/16	.	10	0	0·0
Pot 2	1/8	1/16	.	12	0	0·0
27 Pot 1	1/8	1/8	.	6	5	8·3
Pot 2	1/8	1/8	.	7	4	7·7
28 Pot 1	1/8	1/4	.	5	5	10·0
Pot 2	1/8	1/4	.	5	4	8·0
29 Pot 1	1/8	1/2	.	4	6	15·0
Pot 2	1/8	1/2	.	6	11	18·5

showed that scorching is ameliorated by dressings of this fertiliser. It was considered possible that these scorching effects might be due to (*a*) a deficiency of potash; (*b*) an excess of nitrogen and phosphates over potash. Sand cultures were set up in an attempt to solve this problem. Details of treatment and the results are shown in Tables V to VII.

(3) *Discussion of results.*

The plot observations previously described, indicate that leaf scorch is constantly found on tomato plants liberally manured with nitrogenous and phosphatic compounds, but from which all potassic fertilisers are withheld.

The sand cultures also confirm this, but indicate that potash deficiency is not the only factor affecting leaf scorch. See Tables V to VII.

Leaf scorch did not appear on the untreated plants growing in sand alone. Here the foliage although poorly developed was of a uniform green colour. Further it did not appear on those plants to which ammonium sulphate or superphosphate was given in very small doses. In the absence of added potash, however, larger amounts of ammonium sulphate and superphosphate produced leaf scorch, which increased as the dressings increased. Leaf scorch has not appeared in any experiment where sulphate of ammonia was omitted, but has occurred in the absence of superphosphate. It is concluded that an excess of nitrogen over potash is more effective in causing leaf scorch than is an excess of phosphates over potash. Where dressings of both nitrogen and phosphate are liberal and no potash is given the symptoms of leaf scorch are intensified. These conclusions serve to emphasise the importance of a balanced fertiliser to the tomato crop.

III. Summary.

(1) Blotchy ripening of tomato fruits is the result of malnutrition in respect of potash and nitrogen, especially the former. Phosphates seem to be unimportant in this respect.

(2) The proportion of blotchy fruits may be reduced to less than 1 per cent. by suitable applications of sulphate of potash and sulphate of ammonia.

(3) Blotchy ripening has not been eliminated entirely by manurial treatment. Other factors, probably climatic in nature, play some part in the incidence of this disorder.

(4) Root invading fungi, such as *Colletotrichum tabificum*, interfere with the feeding of the plant and indirectly cause blotchiness of the fruit.

(5) Exposure of the fruits to excessive sunlight causes a type of blotchiness known as "green back" if the nitrogen and potash supply is inadequate.

(6) Blotchy ripening may be accompanied by necrosis of the vascular bundles of the fruit and breakdown of the adjacent tissues with the formation of canals.

(7) A deficiency of nitrogen and potash in the soil in which tomato plants are growing causes distinctive colour changes in the leaf.

(8) The leaves of tomato plants grown in soil deficient in nitrogenous foods, develop pale yellow blotches between the veins. The blotches gradually increase in size until the entire leaf surface is yellow.

(9) Leaf scorch, characterised by a paling and subsequent desiccation of the leaf margin is due to potash starvation.

(10) When potash is absent from the nutrient supply, the severity of leaf scorch increases as the nitrogen and phosphate supply is increased.

(11) Leaf scorch can be eliminated completely by the application of sulphate of potash.

EXPLANATION OF PLATE VI.

Fig. 1. "Green back."
Fig. 2. "Blotchy ripening."
Fig. 3. "Aucuba Mosaic" disease.
Fig. 4. "Stripe" disease.

EXPLANATION OF TEXT-FIGURE 1

Fig. 1. A skinned tomato fruit showing the arrangement of the surface vascular bundles.
Fig. 2. The apical end of a "blotchy fruit," which has been skinned to expose the bundles. The thickened vascular bundles exist immediately below a blotch.
Fig. 3. A vascular bundle from Fig. 2, showing the adjacent parenchyma and neighbouring corky cells (cross shaded).
Fig. 4. Section through a necrotic bundle, showing necrosis *B*, and bordering canals *A*.
Fig. 5. Section through a necrotic bundle showing early signs of necrosis. *A*, Brown cells in neighbourhood of phloem. *B*, Advanced necrosis.

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Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

A.M.W.

SOME OBSERVATIONS ON WHITE CLOVER, AND A METHOD OF DISTINGUISHING BETWEEN THE SEEDS OF WILD WHITE AND DUTCH CLOVER[1]

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(With Plates VII and VIII and 5 Text-figures.)

INTRODUCTION.

The relative importance of Wild White Clover compared to that of Dutch or Commercial Clover is so generally recognised that it need not be emphasised here (15, 4, 14, 7, 8, 9, 3). Mention is first made of cultivating white clover in the eighteenth century in England; the seed was costly owing to the difficulty of harvesting, but it was valued even then on account of its observed permanent quality (16, 4). There is no doubt that during the eighteenth century large quantities of white clover seed were imported from Holland, and the name Dutch came to be used generally for all forms of cultivated and imported white clover seed in contra-distinction to wild white clover seed which is generally accepted as the seed obtained from pastures which have not been cultivated for half a century or more. It is only within the last forty years that such great importance has been attached to the value of wild white clover as a pasture plant on account of its undoubtedly permanent and colonising properties (10). An area of over two square feet of dense herbage is frequently produced by a typical plant derived from one seed of genuine wild white clover, the seed of a typical Dutch clover plant produces a plant with fewer runners or branches and of a "straggly" nature which gradually dwindles away towards the end of the season, whilst the wild white clover is still a dense mass of foliage and is permanent. This great difference in the value of the plants for forage purposes is proved by the results obtained from the preliminary experiments on yield given in Table I below:

[1] The work was carried out at the Welsh Plant Breeding Station, Aberystwyth.

Table I.

Green weight and dry weight of plants, August 1924.

Variety	Green weight per ten plants gm.	Dry weight per ten plants gm.
English wild white	1216	255·5
New Zealand	974	138·6
*American	958	199·9
Irish wild white	840	173·5
English commercial	537	96·4
Dutch	531	104·3
Italian	471	84·9
Danish	456	88·3
Polish	420	82·6
Slovakian	232	43·7
Silesian	—	—

* American plants had a very large number of flower heads which probably added considerably to the green and dry weights.

The above results and the fact that the price of wild white clover soared up to 35*s*. per lb. and is still comparatively high makes it important to distinguish between the seeds of wild white and Dutch clover[1]. But, although the plants differ thus in habit and yield, the seeds of wild white and Dutch clover are so similar that it has been almost impossible even for the expert to distinguish them in the ordinary way; this difficulty is experienced both by the seed merchant and the agriculturist.

DESCRIPTION OF METHODS ALREADY IN USE TO DISTINGUISH BETWEEN SEEDS OF WILD WHITE AND DUTCH CLOVER.

There are several methods generally employed:

The weight of one thousand seeds.

The percentage of seeds which will pass through a sieve of 1 mm. mesh (wild white are considered to be smaller than Dutch).

The chemical test for the cyanophoric reaction.

Occurrence of various weed seeds in the samples.

Discussion of Methods

The average weight of 1000 seeds of Dutch origin is ·66 gm. (·6–·71 gm.).

The average weight of 1000 seeds of wild white is ·50 gm. (·41–·75 gm.).

The weight of 1000 seeds is not a very reliable test as the ranges of weights overlap, size of seed is related to weight, and the weight of

[1] The market price of wild white clover has been seven times and is still three times as great as that of ordinary white clover. (Anderson, T., *Rep. of the Fourth Internat. Seed Test Congress*, 1925.)

1000 seeds of the larger seeded wild type is approximately that of Dutch.

The use of a sieve will not distinguish between a large-seeded sample of wild, and one of Dutch containing a considerable number of small seeds. The chemical test for the cyanophoric reaction, if positive, is said to indicate (not prove) that a given sample is one of wild white clover; if negative, that the sample is not one of wild white clover. Samples of wild white clover seed have been found, however, in which all the individuals tested gave negative results. Two similar wild white clover plants growing together gave, the one a positive, the other a negative result when tested. Similar results were obtained by Sampson(13), and by Ware(16).

The presence of weed seeds of arable land (in small quantities) may indicate that the given sample has been obtained from pasture of a comparatively recent date, but should not on that score alone condemn the sample as one incapable of producing plants of a permanent nature. The seed merchant for the most part has to rely on the guarantee of the grower, or a knowledge of the place of origin of the seed, or a personal inspection of the crop.

The problem has been studied in order to find if there is not a more reliable method and one which will go further to distinguish the seeds of the different varieties of white clover.

The following is a brief account of some of the methods employed and the results obtained.

Method devised to distinguish wild white from Dutch clover seed.

Shape types. An examination of samples of white clover from various sources showed that in any one sample the seeds could be sorted out into several types according to shape. It is possible, according to Capitaine(2), to distinguish the seeds of any variety of plants by their shapes and markings. Seeds from white clover samples were divided roughly into three types called, for the sake of convenience, Type I, Type II, and Type III.

Type I is mitre-shaped, a deep groove or wrinkle separates the radicle from the cotyledons. It is slightly flattened laterally which gives it a somewhat wedge-shaped appearance, the thin end of the wedge is formed by a radicle which runs the whole length of the cotyledons. In front view, *i.e.* looking at the hilum, it is like the shape of a pear drop.

Type II is also mitre-shaped, but the groove or wrinkle between the radicle and cotyledons is absent or very slight, a small notch at the base

of the seed defines the position of the radicle. The seed rolls easily when touched and is not flattened. In front view it is of a more oval and rounded shape than Type I.

Type III. In profile the radicle of Type III is much shorter than the cotyledons and projects slightly. It resembles a human profile with a rather prominent nose and receding chin, it is also grooved and may briefly be defined as the crochet-hook type.

In commercial or Dutch seed samples, Type I either predominates or is present in equal proportions with Type II. In wild white clover samples, Type II predominates. Type III has a somewhat higher percentage in Dutch than in wild white samples. In samples stated to be once grown and from leys up to five years the percentage of Type II appeared lower than in genuine wild white but higher than in the commercial samples. Hand-picked clover from genuine old pasture fifty to one hundred years old was used as a standard of comparison.

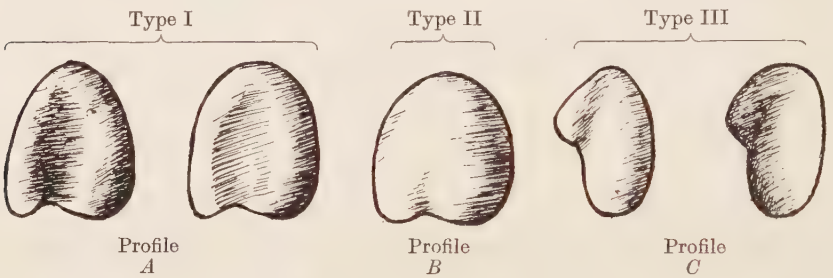


Fig. 1. Drawings illustrating type shapes of seeds in profile. *A*, Type I. *B*, Type II. *C*, Type III.

Size of seed. Accurate measurements by calipers of individual seeds from a number of samples have shown that, although in Dutch clover the large seeds predominate, and the largest are larger than those of wild white clover, there are also in genuine wild white clover samples, seeds of considerable size. Care was taken that the seeds were representative of the sample, and that sufficient measurements were taken to obtain accuracy.

Lodino or Mammoth white clover, which is the largest and coarsest of the white clovers in stem and leaf habit, has a seed as small as that of wild white clover. It was found that when the average ratio of the longest diameter to the shortest diameter was taken, a figure was obtained which correlated with the percentage frequency with which Types I, II, and III occurred in wild white and commercial white seed samples. Results are given in Table II. At least five hundred seeds were sorted out to obtain percentage of shape types; in finding ratio the

averages of 10, then of 20, then of 30 observations were found, and so on, till the ratio remained constant. The number of seeds measured in each sample was from 80 to 150.

Table II.

Shape types and size of seed.

Varieties	Percentage of shape types I	II	III	Long diameter Range	Av.	Short diameter Range	Av.	Ratio L.D./S.D. Range	Av.	Prob. error
Wild white clover hand-picked samples (6)	11	84	5	·9–1·6	1·178	·6–1·0	·795	1·11–2·00	1·46	·0101
Commercial samples (4)	17	73	10	·9–1·5	1·155	·6–1·0	·777	1·11–2·00	1·48	·0107
Cultivated wild (6)	30	61	9	1·0–1·4	1·190	·6–1·0	·785	1·11–2·0	1·52	·0111
Dutch (4)	43	44	13	1·0–1·8	1·260	·6–1·0	·795	1·20–2·16	1·56	·0121
Dutch grown (1)	68	23	9	1·2–1·7	1·320	·6– ·9	·790	1·33–2·0	1·67	·0114
New Zealand (3)	37	53	10	1·0–1·5	1·223	·6–1·0	·773	1·33–2·0	1·58	·0099
Lodino (3)	44	39	17	1·0–1·4	1·190	·6– ·8	·760	1·25–2·16	1·56	·0106
American (3)	29	60	11	·9–1·4	1·163	·5– ·9	·746	1·22–2·00	1·56	·0106

N.B. Figures in brackets give number of samples from which data were obtained in each case.

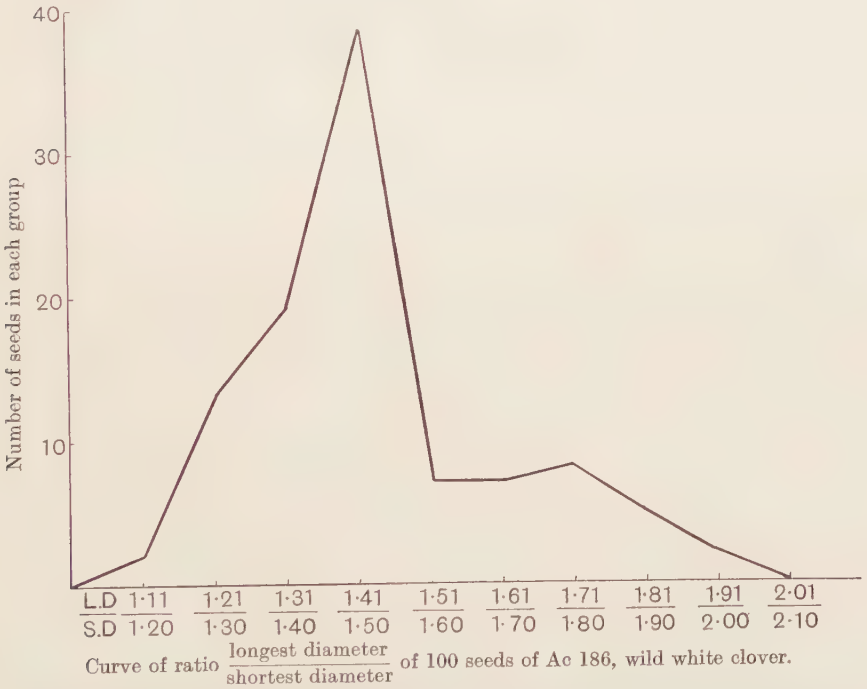
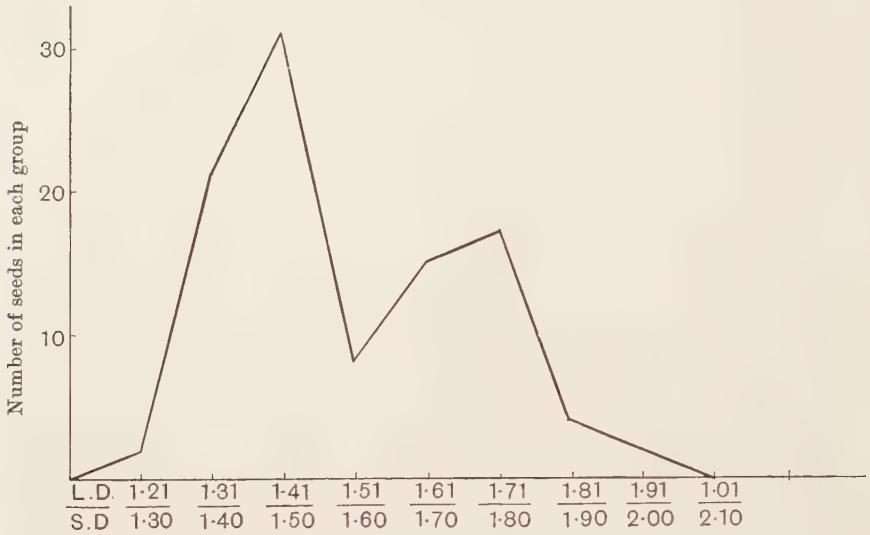
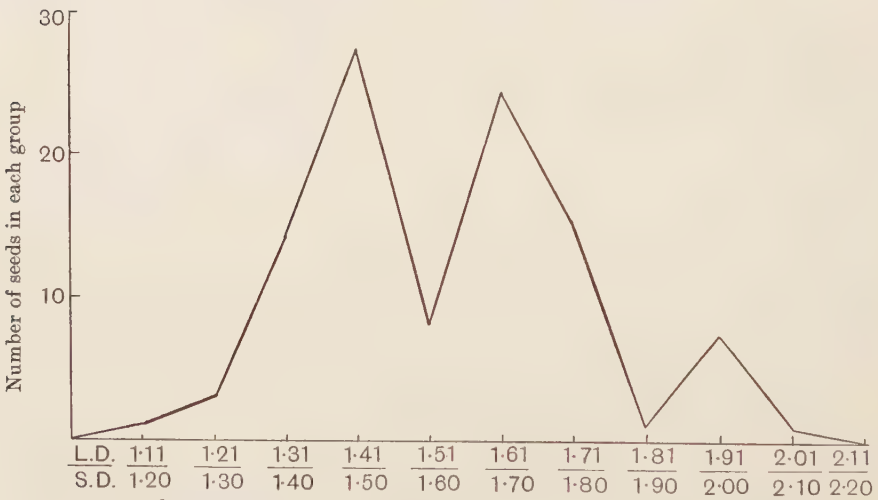


Fig. 2. The curve representing the distribution of ratios of 100 seeds of sample Ac 186 is typical of six such curves which were plotted of seeds of genuine wild white clover samples, and is similar to the normal frequency curve.



Curve of ratio $\frac{\text{longest diameter}}{\text{shortest diameter}}$ of 100 seeds of sample Ac 245 once grown wild white clover.

Fig. 3. This curve representing the distribution of ratios of 100 seeds of Ac 245, which is a sample of cultivated wild white clover, is typical of four such curves which were plotted of samples of cultivated wild white clover. This curve approaches the bimodal type and suggests a mixture of varieties coming in.



Curve of ratio $\frac{\text{longest diameter}}{\text{shortest diameter}}$ of 100 seeds of sample Ac 161 English commercial white or Dutch clover.

Fig. 4. This curve representing the distribution of ratios of 100 seeds of Ac 161, which is a sample of Dutch, is typical of six such curves which were plotted of samples of Dutch clover. This curve is bimodal and indicates a mixture.

Planting and growing of samples of ten nationalities.

Thirty-three samples including ten nationalities were sown in boxes in a cool greenhouse in October, 1923. Observations were recorded of first appearance of cotyledons and time of expanding. Further observations showed distinct differences in size of cotyledons. Some measurements obtained led one to believe that size of cotyledon is related to variety, and would be a character useful in distinguishing wild white clover from the commercial seed (12). Table III gives results from data obtained at seedling stage. As growth continued, the formation of auxiliary shoots and a prostrate habit were characteristic of plants of the wild type, results shown in Table V. Data from final stages of growth are given in Table VI. Notes were made in November, 1924, and again in May, 1925, of the growth and degree of permanency observed of the different nationalities sown in 1923.

Size of seedling. Length of cotyledon multiplied by width gave a fairly constant figure, the area of the cotyledon in the indigenous was found to be less than in the commercial. The length of the petiole of the first foliage leaf was also found to be less in the indigenous than in the commercial. A large number of seedlings, from 50–100, were measured to obtain data which are given in Table III. The results from observations of 20 seedlings from another sowing of seed in May 1925 are given in Table III *a*. Care was taken that the seeds were sown sufficiently thinly so that individual seedlings might have sufficient space and uniform conditions in each box. Further, the seedlings should be collected when the cotyledons are fully mature as they increase in size after they unfold.

Ac 190 average area of 20 cotyledons when first expanded, 5·42 sq. mm.
Ac 190 average area of 20 cotyledons when fully grown, 8·06 sq. mm.

Cotyledon of same plant	When first unfolded L. × B. in sq. mm.	Later L. × B. in sq. mm.	Fully grown L. × B. in sq. mm.
Ac 179	2 × 1	2·5 × 1·5	5 × 2·8
Ac 211	2 × 1	2·0 × 1·2	2·3 × 1·4
Ac 257	2 × 1	2·4 × 1·5	3 × 2·0

In comparing the areas of the cotyledons of different samples it is better to take observations of at least forty seedlings. The probable error of the difference between the average area of 20 cotyledons of Ac 190 wild white and Ac 188 Dutch, when calculated gave the figure 6·5; this figure 6·5 is significant (1) and indicates that the two samples are distinct varieties. In samples where the size of the seedlings varies greatly, as in cultivated wild white and Dutch, a larger number of

observations is necessary in order to obtain a reliable figure for the comparison of samples. The P.E. percentage for 20 observations of Ac 190 was 2·4, and for 50 observations 1·9.

Table III.

Size of seedling, grown October, 1923.

Variety	Area of cotyledon: Range sq. mm.	Area of cotyledon: Average sq. mm.	Length of petiole of 1st leaf: Range mm.	Length of petiole of 1st leaf: Average mm.
Wild white clover, hand-picked samples (3)	3·8–15·2	8·50	3–20	9·76
Commercial samples W. W. C. (6)	4·2–16·0	8·24	4–23	12·32
Dutch (4)	5·4–20·4	12·57	6–28	15·11
Lodino (1)	6·4–26·0	10·05	8–30	18·20
American (1)	6·3–15·0	10·33	7–20	14·60
Danish (1)	11·5–24·8	15·76	12–29	17·50

Table III *a*.

Seedling grown, May and June, 1925.

Wild white, hand-picked samples (4)	3·9–11·2	7·22	5–20	11·22
Commercial samples, wild white (6)	3–15·9	8·60	6–23	10·91
Cultivated wild white (4)	5·9–16·8	8·71	7–19	12·07
Dutch (5)	6·6–18·6	12·17	8–23	14·42
Italian (2)	6·0–10·0	8·04	10–19	14·20
American (2)	5·7–11·2	8·48	9–19	12·35
Danish (1)	7·0–14·0	10·83	9–18	12·9
Polish (2)	6·0–16·0	10·70	10–24	16·77
Bohemian (1)	6·8–13·63	10·07	9–19	13·15
New Zealand (3)	5·4–11·2	7·76	9–23	14·55

The differences between wild white and Dutch clover seeds and seedlings may be summarised thus from the above Tables II, III and III *a*.

Seed measurements.

Variety	Shape types	Long diameter	Short diameter	Ratio $\frac{\text{L.D.}}{\text{S.D.}}$
	Wild White Clover.			
	Type II predominates			
Genuine W. W. C.	Range 80–88 %	Range ·9–1·6 mm.	Range ·6–1·0 mm.	Average 1·46
Commercial W. W. C.	„ 61–81	„ ·9–1·6	„ ·6–1·0	„ 1·48
Cultivated W. W. C.	„ 55–66	„ ·9–1·6	„ ·6–1·0	„ 1·52
	Dutch Clover.			
	Type II only averages 39 %			
Commercial English	Range 38–52 %	Range 1·0–1·8 mm.	Range ·6–1·0 mm.	Average 1·56
Dutch grown	„ 23	„ 1·0–1·8	„ ·6–1·0	„ 1·67

Seedling measurements, grown October, 1923.

Variety	Area of cotyledon	Length of petiole
Wild white	Average 8·5 sq. mm.	Average 9·7 mm.
Dutch	„ 12·57	„ 15·1

Seedling measurements, grown May, 1925.

Variety	Area of cotyledon	Length of petiole
Wild White	Average 7·22 sq. mm.	Average 11·2 mm.
Dutch	„ 12·17	„ 14·4

It will thus be seen that four diagnostic characters have been found which are of use in distinguishing wild white clover from Dutch:

(*a*) The percentage of shape types which occur—80 per cent. of Type II and over—indicate that the sample is one of genuine wild; less than 50 per cent. that the sample is one of Dutch.

(*b*) The ratio of the long diameter is less in wild white than in Dutch.
Average ratio in wild white was 1·46.
Average ratio in Dutch 1·58.

(*c*) Area of cotyledon of seedling is less in wild white than in Dutch.
Average area of cotyledon wild white clover 8·5 sq. mm.
Average area of cotyledon Dutch white clover 12·5 sq. mm.

(*d*) Length of petiole of first foliage leaf is less in wild white than in Dutch clover.
Average length of first foliage leaf wild white clover 9·7 mm.
Average length of first foliage leaf Dutch clover 15·1 mm.

It might be mentioned here that the tests (*a*) and (*b*) were applied to samples submitted for diagnosis; in one case particularly the opinion was soon formed that the sample was not one of genuine wild white clover; this opinion was corroborated afterwards. The sample had been bought at a high price as genuine wild white, but sold at a loss as Dutch.

Cyanophoric Tests.

A number of individual plants of different varieties were tested for the cyanophoric reaction. Individual seedlings were tested when the second or third foliage leaf had appeared.

Summary of results.

Out of 112 tests of sample Ac 254 hand-picked wild white 69 per cent. were positive.

Out of 145 tests of sample Ac 190 commercial wild white 47 per cent. were positive.

Out of 123 tests of sample Ac 236 (representative sample from bulk English wild white 6 per cent. were positive.

Out of 50 tests of sample Ac 209 hand-picked wild white all were negative.

Out of 32 tests of sample Ac 208 hand-picked wild white all were negative.

Out of 28 tests of sample Ac 188 Dutch clover, one plant gave a positive reaction.

Out of 14 tests of Lodino samples all were negative.

Out of 26 tests of Danish (morso) all were negative.

Out of 13 tests of Polish all were negative.

Out of 62 tests of New Zealand 12 plants gave a positive reaction.

Out of 10 tests of American 3 plants gave a positive reaction.

Individuals of one head were tested of Ac 254, in no case did all the individuals of one head give a positive cyanophoric reaction. It will thus be seen that samples of wild white clover vary greatly from those which produce a high percentage of cyanophoric individuals to those which in every case gave a negative result.

Microscopic and Histological Work.

The size and arrangement of cells of the testa give diagnostic characters which are used as a test in pharmacy to distinguish between varieties of leguminosae (6). Several methods of imbedding the seeds of wild white and Dutch clover were tried before satisfactory sections suitable for accurate cell measurements by micrometer eyepiece were obtained. The size and the shape of the epidermal and hypodermal cells

Table IV.

Some measurements of the cells of the testa.

Testa	Ac 164 New Zealand	Ac 208 (Irish Wild)	Ac 162 English Commercial
Longitudinal sections of the palisade layer of epidermis			
Epidermal cells			
Length	Average 38μ	Average 44μ	Average 37μ
Width	„ 10	„ 8	„ 8
L.S. hypodermal cells			
Hypodermal cells			
Length	„ 9	„ 7	„ 6
Width at apex	„ 21	„ 20	„ 20
Width at base	„ 28	„ 26	„ 27
Depth of "light line" from surface	„ 9	„ 8	„ 7
Thickness of cuticle ...	„ 1	„ 2	„ 2

vary towards the hilum; it was found necessary, therefore, to take sections of the same part of the seed-coat in each sample of the seed examined. Table IV gives results of measurements obtained.

Cell measurements of the testa of seeds of any sample vary *inter se*, and so far do not show sufficient differences between samples to afford diagnostic characters of value in distinguishing the seed of wild white from that of Dutch clover.

Presence of Nodules.

Attention was drawn to this point and some of the plants were compared as to degree of "nodulosity." Of those examined Ac 214, a New Zealand sample, showed highest; Ac 185, Morso, next; Ac 179, Dutch, a fair amount; Ac 209 and 195, Irish, low; Ac 162, English commercial, almost nil. The investigation of nodule formation might afford some further diagnostic differences in varieties of white clover.

The nodules on wild white appeared to be smaller and more numerous, those of Dutch larger, and of a more sac-like shape, and fewer in number.

Second Stage of Growth.

Comparison of habit of growth of wild white and Dutch clover.

Seedlings of the ten nationalities already sown were planted out in large wooden boxes about two inches apart. The boxes held sixty plants, and the second or third foliage leaf had appeared before the seedlings were transplanted. A marked characteristic of good types of the wild white plant was the prostrate habit and close rosette form at a very early stage of growth, together with the formation of a considerable number of axillary shoots. This was particularly striking in two samples, one Ac 209 a hand-picked sample from the coast of Donegal, another Ac 190, a commercial sample of wild white clover of Kentish origin; this tendency to form axillary shoots at an early stage was also marked in the New Zealand samples. In contrast at the same stage was

Table V.

Number of axillary shoots on individual plants.

Station No. and variety	No. of plants observed	No. of axillary shoots on individual plants
Ac 209 Irish wild white	120	5–6
Ac 195 Irish wild white	120	5–6
Ac 190 English wild white	120	3–5
Ac 214 New Zealand wild white	120	4–6
Ac 162 Commercial or Dutch	120	0–2

the upstanding form, leafy luxuriance and small development of axillary shoots of Dutch and commercial samples (Plate VIII, figs. 5, 6, 7). A few results are given in Table V.

Final Stages of Growth.

A number of boxes of wild white and Dutch clover including all the nationalities were selected, and the plants from these were planted out in April in rows about two feet apart.

The growth of a typical plant of wild white clover was characterised by a further development of stolons or axillary shoots; on many plants up to twenty were counted. These main stolons develop strong roots at the nodes and bear secondary and generally tertiary shoots which also develop their own root systems at a fairly early stage. In many plants the apex of the main stolons showed a tendency to turn towards the ground near the growing point after about the eighth node had been reached; many of the stolons actually penetrated the ground to a depth of half an inch or more, turned white and formed a strong branching root system before again emerging. It was difficult sometimes to trace a stolon back to the parent stock, so closely did it hug the ground by a series of roots at almost every node. Several plants of wild white clover which had appeared on a bare spot of ground where none had been planted were carefully dug up, stems were found at a depth of three to six inches, at the end of these main stems pieces of the original stem decayed at one end, sometimes at both ends were found (Fig. 5).

This is eventually what happens: at an earlier stage as described the stolons develop strong roots which supply the growing parts with food so that the stolon or runner if severed at the base can live independently of the parent plant. The runners decay at their base near the centre of the rosette but not before a large area of ground is colonised, the part of the stolon which is under the ground can then be drawn deeper by the contraction of the roots at that spot.

The Dutch type forms a striking contrast, the stolons are fewer, about four to five in number, with little development of secondary shoots, a few stolons may attach themselves feebly to the ground, but they only form weak propagants, the greater number of the plants in general dwindle away or almost disappear towards the end of the season. Some plants possess buds and a few young shoots at the apex of the root-stock which may last for a year or two, but the plant eventually dies.

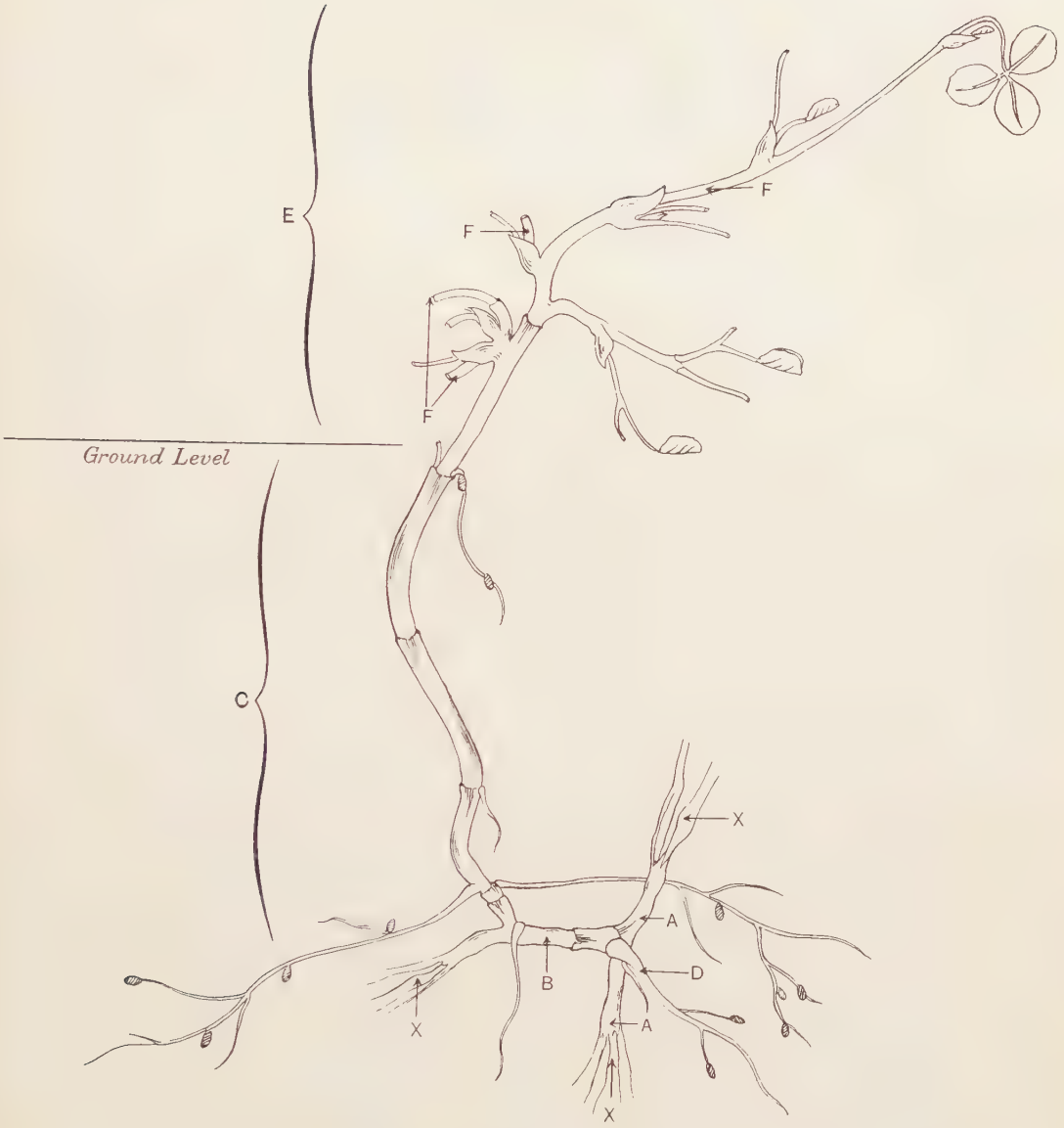


Fig. 5. Vegetative shoot of wild white clover showing parts of original runners from which it has arisen. *A*, Part of original runner decayed at both ends, *X*. *B*, Shoot arising from *A*. *C*, Shoot arising from *B*. *D*, Largely developed root of *A*. *E*, Aerial parts of plant. *F*, Aerial shoots on *E*. *X*, Decayed parts of shoots *A* and *B*.

Maturity of Plants.

Further observations were recorded and data collected during the period of greatest growth. Differences in time of flowering, position of flowers, and number of flowers per head are given in Tables VI *a* and VI *b*.

Table VI *a*.

Comparative observations made in June, 1924.

Variety	Percentage of plants in flower 3rd week in June	Percentage of plants in flower 4th week in June	Nodes at which flowers usually appear	Length of peduncle cm.	No. of nodes per longest stolon	Length of internodes cm.	Greatest length of petiole observed cm.
English wild white	0·2	0·4	4th to 8th 6th frequent	3·5– 5·5	6–10	1·2–3·0	4·5
English commercial	5·0	10·0	1st to 6th	4·5– 9·0	4–7	1·0–3·5	6·0
New Zealand	11·0	21·0	1st to 4th	6·0–10·0	7–8	1·5–4·5	7·0
American	0·0	8·3	4th to 6th	6·5– 7·0	7–8	1·5–3·0	6·0
Danish	3·8	30·0	1st to 4th	4·5– 9·0	4–7	1·5–2·8	5·0
Italian	0·0	1·3	5th to 7th	—	4–7	1·2–2·8	7·0
Polish	14·4*	26·0	1st to 5th 1st frequent	4·0–12·0	4–6	1·0–2·0	5·0
Irish indigenous	0·7	2·0	4th to 6th	3·5– 4·0	6–7	1·0–2·8	3·5
Slovakian	13·0	25·0	1st to 7th 1st frequent	5·0– 7·0	5–6	1·5–2·5	6·0
Silesian	3·0	32·0	1st to 7th	7·0– 8·0	4–5	1·5–2·5	4·5
Dutch	5·0	13·0	1st to 8th	6·0– 8·0	6–8	1·5–2·5	6·5

* Some flower in first week.

Table VI *b*.

Comparative observations made in July and August, 1924.

Variety	Percentage of plants without flowers in bloom	Av. No. of flower heads per plant in bloom	No. of mature heads per plant Range	Av. No. of heads per plant (10 plants)
English wild white	45	2·8	1–49	6·6
English commercial	1	8·8	3–74	20·7
New Zealand	8	7·6	4–62	29·4
American	14	6·7	6–66	32·7
Danish	21	4·5	3–54	17·5
Italian	32	1·7	2–17	6·0
Polish	0	10·0	3–29	16·0
Irish	47	1·5	0– 3	2·0
Silesian	6	13·0	—	—
Slovakian	5	9·0	—	—
Dutch	0	11·0	5–46	25·3

From above, Tables VI *a* and VI *b*, it is seen that wild white clover produces much fewer flower heads per plant than Dutch clover, and it is later in flowering.

Flower heads in wild white are borne on older parts of the plant. Wild white shows greater vegetative activity, the stolons reach a tenth node, those of the Dutch an eighth node.

Length of petiole and peduncle are shorter in wild white than in Dutch.

Wild white clover 4·5 cm. (length of petiole). Dutch 6·5 cm. (length of petiole).

Wild white clover 5·5 cm. (length of peduncle). Dutch 9·0 cm. (length of peduncle).

Comparative Observations on the Growth and Permanence of Plants of Different Nationalities.

The results of close observations made on each sample at the end of one year's growth and also at the end of two year's growth may be summed up thus: Indigenous wild white clover seed produced plants of the best type from an economic point of view. Even before the end of the first year these plants had formed a dense mat of herbage colonising the ground in all directions. They did not show any ill effects from frost and had already established themselves permanently in the ground.

New Zealand clover seed also produced plants of a permanent type but of a larger and coarser habit which did not cover the ground as densely as the indigenous. American seed from virgin soil produced plants with some degree of permanency but of a type inferior to indigenous. Many plants are of the straggly type.

Danish seed produced plants with some degree of permanency. About 25 per cent. were good plants. They differ from the indigenous in forming more of a bunch and do not colonise the ground to the same extent.

The English commercial, Polish, Silesian, Slovakian and Dutch produced plants the majority of which, even in the first year, showed signs of disappearing.

Lodino seed produced plants of the largest leaved and coarsest type, they did not stand frost well and suffered from attacks of slugs; the majority were not permanent.

A point which has significance is the fact that plants of the Dutch type were found amongst those grown from samples of wild white clover, and some plants of the wild type amongst those of Dutch origin. In

addition to this many plants intermediate in forms between the extreme type were to be found.

Permanence in White Clover.

From observations of the habit of growth from seedling stage to maturity the conclusion was formed that permanency in wild white clover is obtained by a strongly developed power of vegetative propagation. At an early stage numerous axillary shoots are formed with a well-developed root system, at a later stage the stolons are so firmly rooted that any part if severed from the parent plant is capable of leading an independent existence. It has already been described how these stolons penetrate the ground, give rise to a series of secondary and tertiary shoots which colonise the ground to a considerable extent in all directions, eventually decay at the base near the centre of the rosette and so break their connection with the parent plant.

In Dutch clover the stolons, fewer in number, "straggle" along the ground and form only a few weak propagants. A few young shoots at the apex of the root-stock may last for a season or two, but the plant soon dies. Evidence from early records shows that large quantities of white clover were imported into England and grown there, and that this clover was of the non-permanent type(16).

Cultivated wild white clover.

There is a popular belief that wild white clover cultivated on arable land for three to four generations loses its permanency, and becomes then only comparable in value to Dutch clover(4). If, as seems most probable, permanency is a Mendelian character, an explanation of the apparent degeneracy of wild white clover when cultivated is possible. In old pastures the less permanent types of wild white clover have disappeared because they can scarcely ever sow themselves(17). Seeds from these pastures sown on arable land will produce plants, some most probably of the less permanent strain which will mature earlier and produce a large number of seeds. These when sown and again harvested will form an increasingly larger proportion of the seed harvest so that the third or fourth generation of seed will produce a great number of the non-permanent type of plants. Selection in this direction will continue till the majority of the plants will be of the non-permanent type(15). Further, since white clover is normally self-sterile, there is every possibility that genuine wild white clover when grown on arable land will be cross-fertilised by plants of Dutch clover. These plants may

be growing in the neighbourhood, or some of them may already be present in the same soil. It is very possible that loss of permanence in cultivated white clover is caused by unintentional selection by the grower in the direction of the non-permanent types, evidence was obtained by growing the samples that plants of the less permanent type are present even in the good samples of genuine wild white clover from old pasture; further, that selection in the direction of the non-permanent types is increased by cross-fertilisation with non-permanent types in the neighbourhood. It has been found also by other workers (16) that wild white clover can be cross-fertilised by different varieties of Dutch or commercial white clover.

Dutch clover dies out within the first few years of growth because it has not the strongly developed power of vegetative propagation. The reason sometimes assigned for lack of permanence in Dutch clover is greater susceptibility to disease, *e.g.* eelworm, *Tylenchus dipsaci*, the cause of Stem Disease (5, 16). None of the plants of white clover which were under observation showed signs of Stem Disease.

SUMMARY.

1. Diagnostic characters which have been found of use in distinguishing samples of wild white from Dutch clover seed:

(*a*) Frequency with which certain shape types of seed occur in each sample.

(*b*) Average ratio of long diameter of seed to short diameter.

(*c*) Area of cotyledon.

(*d*) Length of petiole of first foliage leaf.

2. All samples, all individuals of one sample, or of one head of wild white clover, do not give a positive reaction for the cyanophoric test.

3. Further diagnostic characters at a later stage of growth, (*a*) prostrate habit, (*b*) formation of axillary shoots, are characteristic of wild white clover.

4. Permanence in wild white clover is due to a strongly developed power of vegetative propagation. Dutch clovers are not permanent as they lack this power to different extents.

5. Loss of permanence in cultivated white clover is due to unintentional selection of the non-permanent type (by the grower), and to cross-fertilisation by the non-permanent or Dutch plants growing in the neighbourhood.

Acknowledgment is gratefully made to Prof. Bateson, London, for his interest and encouragement throughout the work; to Prof. Stapledon for his help in giving all facilities for the work at the Welsh Plant Breeding Station, Aberystwyth. Thanks are due to Captain R. D. Williams, B.Sc., for assistance in the genetical work; to Mr J. W. Watkins for the cultural work and manipulation of the bees, and to other members of the Staff at Aberystwyth, and also to Prof. Small, Queen's University, Belfast.

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Fig. 3.

Fig. 4

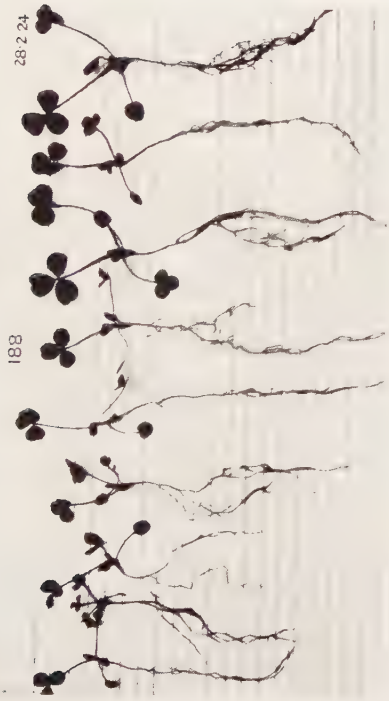

Fig. 1

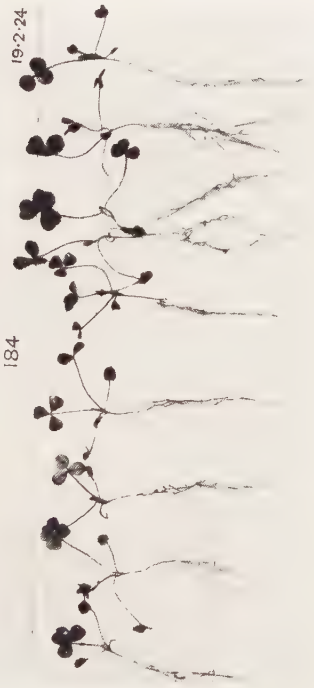

Fig. 2.

Fig. 6.

Fig. 7.

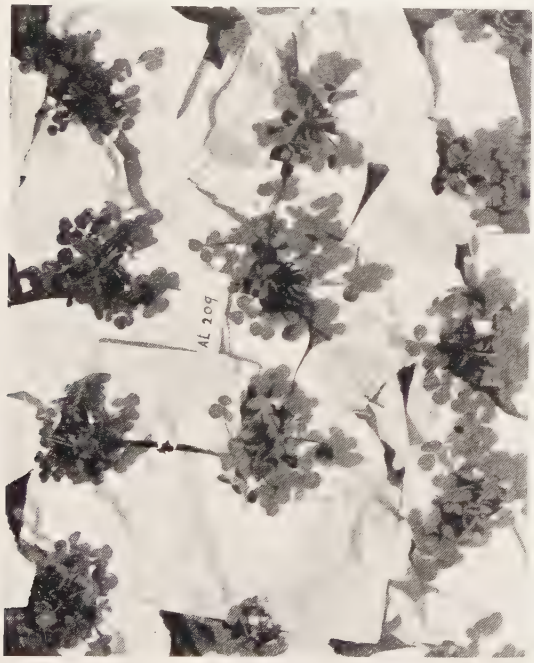

Fig. 5.

EXPLANATION OF PLATES VII AND VIII

Fig. 1. Seedling stage English commercial white clover.
Fig. 2. Seedling stage Polish white clover.
Fig. 3. Seedling stage wild white clover from very old pasture.
Fig. 4. Seedling stage wild white clover, small-leaved type. N.B. Scale is same in all, lines are 5 cm. apart.
Fig. 5. Second stage of growth showing rosette form and prostrate habit of the wild type.
Fig. 6. Second stage of growth showing upstanding leaves and "straggly" habit of Dutch type.
Fig. 7. Second stage of growth showing the extreme type of Dutch.

N.B. Photos were taken about the same date, white paper was put in to make the plants show up against the soil in the boxes.

(*Received January 5th*, 1926.)

WART DISEASE OF POTATOES: THE DEVELOPMENT OF *SYNCHYTRIUM ENDOBIOTICUM* (SCHILB.) PERC., IN "IMMUNE" VARIETIES

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(With Plate IX.)

A method has been described in an earlier paper(1) whereby the immunity or susceptibility of potatoes to Wart Disease can be tested in the laboratory. Living warts bearing summer sporangia are placed in close contact with the young growing shoots to be tested, and are kept in a wet condition. The shoots of susceptible varieties show signs of infection and develop young warts within a few weeks while no warts are observed on immune varieties.

Subsequently it was noticed that small protuberances and surface irregularities resembling the earliest symptoms of infection sometimes appeared on the shoots of certain varieties that are officially certified as immune. These abnormal growths did not seem to develop further and in no instance produced the characteristic warts observed in susceptible varieties. Microscopic examination showed that infection by the fungus *Synchytrium endobioticum* had taken place. Its development to the stage when the sorus contained a number of summer sporangia was observed in the "immune" varieties Aberdonian, Ben Cruachan, Glenmaid, Katie Glover, Kerr's Pink and White City and in some cases the empty sorus which had apparently liberated the summer sporangia was found. Plate IX shows photographs of the sorus in the varieties mentioned. Further development of the parasite, that is, reinfection by zoospores of summer sporangia and subsequent formation of winter sporangia, has not as yet been detected. Certain "immune" varieties other than those mentioned showed no signs of infection, when tested by this method.

The possibility that the apparent infection of "immune" varieties can be explained by the presence of susceptible rogues does not appear likely because the infected "immunes" differed from susceptible varieties

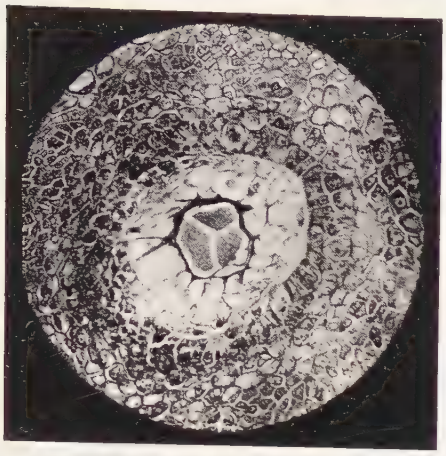

Fig. 1.

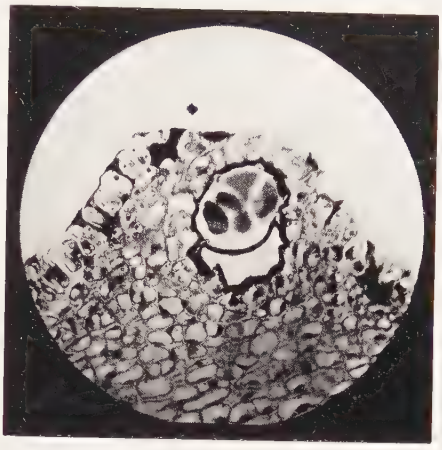

Fig. 2.

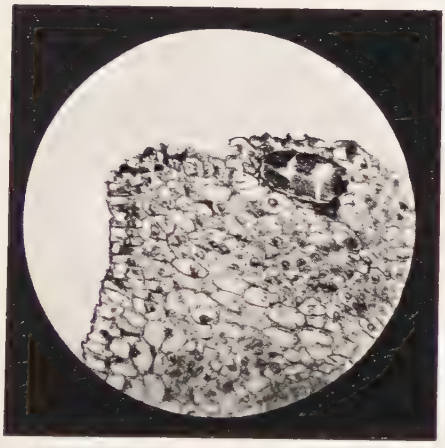

Fig. 3.

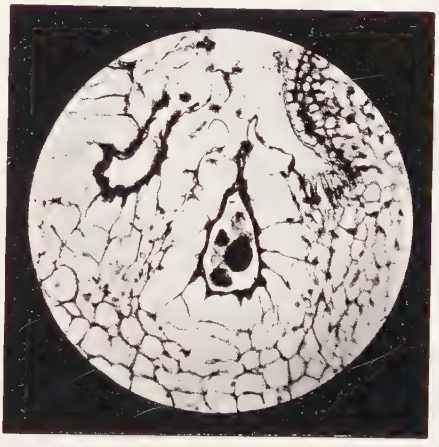

Fig. 4.

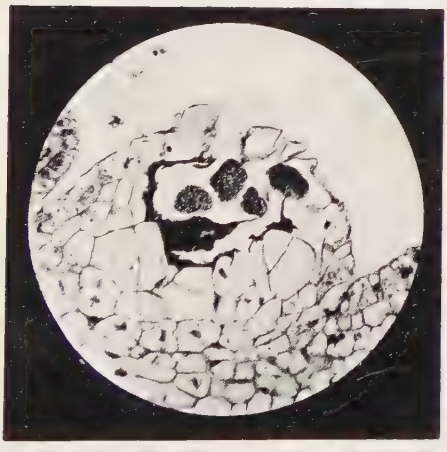

Fig. 5.

Fig. 6.

GLYNNE.—Wart Disease of Potatoes. (358—359.)

in that the incipient infections never developed into actual warts in the former while in susceptible tubers, warts appeared a week or two after the first signs of infection. Further, the tubers were obtained from reliable sources[1] and five typical specimens of each variety were selected for testing. Infections were found on the shoots of several tubers of Aberdonian, Katie Glover and White City but of only one tuber of the remaining varieties.

These tests are being repeated and immune tubers showing signs of infection will be planted and their variety retested. Work is in progress to determine (*a*) which immune varieties are attacked, (*b*) whether winter sporangia are produced, and if not, (*c*) the causes which check the development of the parasite.

[1] Aberdonian, Ben Cruachan, Glenmaid, White City were obtained from Mr H. Bryan, Potato Testing Station, Lathom, Ormskirk, and Kerr's Pink from Mr A. W. McAlister, Dumfries, Scotland, and Katie Glover from Messrs Dobbie and Co., Edinburgh.

EXPLANATION OF PLATE IX.

Fig. 1. Katie Glover. A sorus showing three sporangia. The section is transverse to the host cell. There is some plasmolysis. Stained safranin. Panchromatic plate. Green filter. × 150.

Fig. 2. White City. A sorus showing five sporangia in the sorus and the empty prosoral membrane below. The section is longitudinal to the host cell. Stained Flemming's triple. Panchromatic plate. Green filter. × 150.

Fig. 3. Kerr s Pink. A sorus showing five sporangia. The section is probably oblique to the host cell. Stained Flemming's triple. Ortho process plate. × 150.

Fig. 4. Ben Cruachan. A sorus containing summer sporangia and an empty sorus. The section is longitudinal to the host cell. Stained iron-alum haematoxylin and orange G. Ortho process plate. × 150.

Fig. 5. Aberdonian. A sorus, either broken or about to liberate four summer sporangia. Part of the empty prosoral membrane is seen below the sorus. The section is longitudinal to the host cell. Stained iron-alum haematoxylin. Ortho process plate. × 150.

Fig. 6. Glenmaid. Empty sorus after liberating summer sporangia. The section is longitudinal to the host cell. Stained iron-alum haematoxylin. Ortho process plate. × 150.

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ERRATUM.

In Reference (2), Table IV, p. 26. The sentence "Stain in 2·5 % methylene blue aq." *should read* "Stain in 0·25 % methylene blue aq."

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STUDIES ON THE LUCERNE NODULE ORGANISM (*B. RADICICOLA*) UNDER LABORATORY CONDITIONS

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(With Plates X and XI and 3 Text-figures.)

Introduction.

Since Löhnis and Smith (1) made certain observations on the life-history of some important soil bacteria, pointing out the existence of morphological variations which they held to constitute life-cycles, Bewley and Hutchinson (2) studied the different characteristic stages in the development of the nodule organism (*B. radicicola*) under cultural conditions. They showed that the life-cycle proceeded in five successive stages, namely, (1) pre-swarmer stage (small non-motile coccus), (2) large non-motile coccus, (3) swarmer stage (motile coccus), (4) rod stage (motile), and (5) rod stage of high vacuolation. They observed these stages under various conditions of aeration, temperature and food supply in artificial media.

Scope.

The investigation recorded in this paper was undertaken to ascertain whether the various stages observed by Bewley and Hutchinson form a true life-cycle of the organism and whether any form may pass into the other under varying cultural conditions. The observations were made both in (1) liquid, and (2) solid media, and also in (3) soil. The study of morphological variations of the organism is essential for an understanding of its physiological behaviour.

(*a*) *In liquid media.*

Methods. The following liquid media were used for the cultivation of the organism:

(1) Distilled water.
(2) "Ashby" mineral salt solution.
(3) Soil extract.

(4) Soil solution.
(5) Straw extract.
(6) Lucerne top extract.
(7) Lucerne root extract.
(8) Bean seedling extract.

"Ashby" (3) solution consisted of:

Magnesium sulphate	... ...	0·2 gm.
Sodium chloride	... ...	0·2 ,,
Mono-potass. phosphate	...	0·2 ,,
Calcium sulphate	... ...	0·1 ,,
Calcium carbonate	... ...	0·2 ,,
Water (distilled)	... ...	1000 c.c.

The soil extract was made by steaming 250 gm. of 3 mm. sieved garden soil with 200 c.c. of distilled water for two hours. The liquid was filtered through a Berkfeld filter candle.

The soil solution[1] was some that had been obtained by a displacement method. Garden soil passed through a 3 mm. sieve was found to contain 20 per cent. moisture. 4 kg. were packed by gentle ramming of 200 gm. portions into a metal cylinder 4 in. in diameter, having a grid over the outlet at the base. The top of the soil column was then covered with washed sand and distilled water from a large aspirating bottle allowed to form a head of 2–2½ in. It was calculated that 75 per cent. of the total soil moisture could be taken as undiluted soil solutions. (Parker (4).) The solution came through as a light amber-coloured fluid.

The straw extract had been prepared as follows. Milled oat straw passing a fine-meshed wire sieve (50 divisions to the inch) was boiled with distilled water for an hour, allowed to stand for 24 hours and filtered through cotton wool. The dry matter in the filtrate was determined and adjusted to 0·4 gm. per cent.

The extracts from lucerne top and roots and from bean seedlings were prepared as follows. The material was pounded in a mortar and boiled with water for an hour. After macerating 24 hours, the liquid was filtered through cotton wool, and diluted to give 0·2 per cent. dry matter.

1 c.c. of the bacterial suspension from a 14-days' old culture made with 10 c.c. of sterile water in a test tube was added to each of the Erlenmeyer flasks containing 100 c.c. of the sterile liquid media. The

[1] The author is indebted to Mr P. H. H. Gray for making the soil solution and straw extract.

flasks were incubated at 25° C. Examination of the drops (and also of slides stained with carbol fuchsin in doubtful cases) after various periods, gave the following results.

Results.

Table I.

Showing predominant forms observed at intervals in liquid media.

(Dominant forms are mentioned first where two stages are noted.)

Liquid media	3 days	6 days	9 days	14 days	30 days
Distilled water	Short and banded rods	Short and banded rods	Banded rods	Banded rods	None
Ashby solution	Short and banded rods	Short and banded rods	Cocci and short rods	Banded rods	Banded rods
Soil extract	Motile cocci and short rods	Short rods	Motile cocci and short rods	Banded rods and cocci	Banded and rods
Soil solution	Motile cocci and short rods	Short rods	Short and banded rods	Banded rods	Banded rods
Straw extract	Short rods and cocci	Short rods	Short rods	Short rods	Banded and short rods
Lucerne top extract	Short rods and cocci	Short rods and cocci	Short rods	Banded rods	Banded and short rods
Lucerne root extract	Short rods and cocci	Short rods and cocci	Short rods and cocci	Banded and short rods	Banded and short rods
Bean seedling extract	Short rods and cocci	Short rods and cocci	Short rods	Banded and short rods	Banded and short rods

As the conversion of one stage to another appeared to be effected in course of a short time, the experiment was repeated and observations were made at frequent intervals during the first 24 hours.

Table II.

Showing predominant forms observed at intervals.

(Dominant forms are mentioned first where two stages are noted.)

Liquid media	3 hours	6 hours	9 hours	12 hours	24 hours
Distilled water	Banded rods	Short and banded rods	Short and banded rods	Banded rods	Banded rods
Ashby solution	Banded and short rods	Banded and short rods	Banded rods and cocci	Banded rods and cocci	Cocci
Soil extract	Banded and short rods	Banded rods and cocci	Cocci	Cocci and short rods	Cocci and short rods
Soil solution	Banded and short rods	Banded rods and cocci	Cocci and short rods	Cocci and short rods	Cocci and short rods
Straw extract	Banded rods	Banded and short rods	Short rods	Short rods	Short rods
Lucerne top extract	Banded and short rods	Banded and short rods	Short rods	Short rods and cocci	Short rods
Lucerne top extract	Banded and short rods	Banded and short rods	Short rods	Short rods and cocci	Short rods
Bean seedling extract	Banded and short rods	Banded and short rods	Short rods	Short rods and cocci	Short rods

From these observations on the effect of various liquid media on the predominance of a particular form, it is noted that soil extract induces the motile stage earlier than any of the other liquid media tested in the experiment. Motility was not observed in the first 24 hours.

An experiment was then made to ascertain whether the addition of certain mineral salts to the soil extract would have any marked influence upon the organism in inducing morphological variations.

Method. To 100 c.c. of soil extract the compounds, as given in Table III, were added. After sterilisation 1 c.c. of a bacterial suspension was added to each of the flasks and the cultures then incubated at 25° C.

Results. The results of periodic examination up to the 14th day are recorded in the following table.

Table III.

Compound added to 100 c.c. soil extract Weight in gm.	Predominant form of organism after					
	18 hours	1 day	3 days	7 days	10 days	14 days
	Banded and short rods	Short rods and cocci	Banded rods and cocci	Motile cocci	Cocci and short rods	Banded rods and cocci
0·1 pot. sulph.	Banded and short rods	Banded and short rods	Short rods and cocci	Short rods and cocci	Cocci and short rods	Banded rods
0·1 calcium sulphate	Banded and short rods	Banded and short rods	Short rods and cocci	Short rods and cocci	Cocci and short rods	Banded rods
0·1 di-pot. phosphate	Banded and short rods	Banded and short rods	Short rods and motile cocci	Short and banded rods	Banded and short rods	Banded rods
0·1 mono-pot. phosphate	Banded rods	Cocci and short rods	Motile cocci and short rods	Short and banded rods	Short rods	Banded and short rods
0·1 calcium phosphate	Cocci and short rods	Motile cocci and short rods	Motile cocci and short rods	Short rods	Banded and short rods	Banded and short rods
0·1 calcium carbonate	Banded and short rods	Banded and short rods	Banded and short rods	Short rods	Banded rods	Banded rods
0·5 calcium carbonate	Banded and short rods	Banded and short rods	Banded rods and cocci	Cocci and banded rods	Banded rods	Banded rods

The addition of calcium phosphate to the soil extract medium caused the motile forms to appear within 24 hours. The other forms of phosphate have also a favourable action in hastening the motile stage. The finding of Bewley and Hutchinson that phosphates hasten the appearance of the "swarmer" stage is thus confirmed; but their conclusion that the presence of excess of calcium carbonate influences the formation of the "preswarmer" stage is not in accordance with the observation made in the present investigation. It is observed that the organisms growing in a solution containing calcium carbonate exhibit a tendency towards the formation of banded rods.

(*b*) *In solid media.*

Method. In view of the marked effect of soil extract on the life-cycle of the organism a number of soil extract media were prepared with the addition of saccharose, mannite, phosphate, lucerne seedling and bean extract. The sloped media were all inoculated from the same culture and incubated at 25° C.

In preparing slides from the culture grown on different media, erythrosin dye was found to give the best result. The cells were well stained and in the case of vacuolated stage the chromatin particles were clearly distinguished from the cell wall. This stain has also been used for the staining of flagella. The method adopted for the latter technique is as follows.

A drop of suspension from a fresh culture is placed at one end of the slide with a platinum loop and the slide is tilted in order to allow the drop to spread over it. The film is dried in an oven at 45° C. and fixed with absolute alcohol. After the alcohol has evaporated the film is flooded with 5 per cent. erythrosin stain dissolved in 70 per cent. alcohol, which is allowed to act for 10 minutes. After washing off the alcoholic stain the slide is restained with 5 per cent. phenolic erythrosin (1 gm. of stain dissolved in 5 per cent. phenol) for another 10 minutes. By this method, the flagella of the lucerne organism were successfully stained, and in the absence of the precipitate usually obtained from the use of mordants, the flagella and cell-wall of the organism were clearly shown.

Examination of cultures grown on favourable media shows that motile forms can arise from the banded rod stage. The darkly stained chromatin bodies, when still within the vacuolated cell, are observed to

Table IV.

Showing the predominant forms after various periods.

Media	1 day	2 days	3 days	7 days	14 days
Soil extract agar	Banded and short rods	Banded and short rods	Short rods and motile cocci	Short and banded rods	Banded rods
Soil extract saccharose agar	Banded and short rods	Motile cocci and short rods	Motile cocci and banded rods	Short rods	Short rods
Soil extract mannite agar	Banded and short rods	Motile cocci and short rods	Motile cocci and banded rods	Short rods	Short rods
Soil extract saccharose agar (with phosphate)	Banded and short rods	Motile cocci and banded rods	Motile cocci and banded rods	Short rods	Short and banded rods
Soil extract Lucerne agar	Banded and short rods	Cocci and short rods	Short rods	Short and banded rods	Short and banded rods
Soil extract bean agar	Banded and short rods	Cocci and short rods	Short rods	Short and banded rods	Short and banded rods

have flagella, and they eventually emerge as motile cocci (swarmers). The phenomenon has been described by Grieg Smith and is confirmed by the present author. The "preswarmers" of Bewley and Hutchinson are thus not a necessary transitional stage in the production of "swarmers.'

Results. The results of the observation of various stages at different periods are shown in the following table.

It is to be noted that while the media containing soil extract alone induce the production of motile forms, the addition of saccharose, mannite or phosphate greatly hastens their appearance.

(*c*) *In soil.*

Technique. The morphological changes observed in the liquid and the solid media appear to confirm, in the main, the existence of the stages in the life-cycle of *B. radicicola* as described by Bewley and Hutchinson, and it remained to study the behaviour of the organism in soil. But the problem was to obtain a stain which would colour the bacteria without staining the colloidal and other soil particles. Following the suggestion made by Conn (5), Winogradsky (6) developed a technique which is found suitable for the purpose. A smear prepared from a suspension of unsterilised soil is allowed to dry upon the slide and fixed for two minutes in absolute alcohol. After the alcohol has evaporated the preparation is stained with 1 per cent. erythrosin dissolved in a 5 per cent. phenol solution. The stain is allowed to act for 10 minutes and is then washed off with water. The organisms are distinguished from the soil particles by their pink colour.

Working with sterile soil to which a pure culture of *B. radicicola* had been added, it was found necessary to modify the above technique in order to obtain a clearer outline of the bacterial cells. After washing off the phenolic erythrosin, the slide is flooded with 2·5 per cent. erythrosin dissolved in water. The aqueous stain is allowed to act for 10 minutes. With this modification the bacterial cells are clearly defined, enabling one to observe various morphological changes through which the organism passes.

With the technique above described, the life of the bacteria in the soil under varying conditions of aeration and temperature and in soils of varying manurial treatment, was investigated. All the stages described by Bewley and Hutchinson were seen and, in addition, it was observed that the organisms frequently group themselves in colonies consisting of large cocci, attached to masses of colloidal matter.

Effect of aerobic and anaerobic soil conditions on the organism.

Bewley and Hutchinson, by growing nodule bacteria on soil extract mannite agar under anaerobic conditions, made qualitative observations to ascertain the various stages in the life-cycle of the organism at different intervals of time. It was not however, known whether the changes observed in artificial media would take place in the soil. In view of their conclusion that "any deviation from strictly aerobic conditions exercises a prejudicial effect on the growth of the organism," it seemed important to investigate both qualitative and quantitative changes that might occur when the organisms were allowed to grow on sterile soil under aerobic and anaerobic conditions.

Method. The work was carried out with Rothamsted garden soil which had been passed through a 3 mm. sieve; the moisture content was made up to 20 per cent. Ten grams of the soil were put into each of 17 large test tubes of equal diameter and autoclaved twice for half an hour at 15 lbs. pressure. Two drops of bacterial suspension in distilled water made from a 9 days old culture on root extract saccharose agar were added to each 10 gm. of soil, which was then stirred with a sterile glass rod to effect thorough distribution of the organism. In order to obtain an estimate of the number of organisms thus introduced, platings were made from a tube immediately after inoculation. Eight tubes were then sealed with paraffin wax over alkaline pyrogallol. All the tubes were incubated at 25° C. Moisture lost in the aerobic tubes was made up by the addition of the required amount of sterile distilled water at intervals of three days.

Plates were poured at intervals of 3, 7, 14 and 30 days, and slides were prepared on each occasion to determine the percentage of the various stages of the organism. This was carried out by the method described by Thornton and Gangulee. At the 30th day the conditions were reversed and the experiment was continued for another 30 days.

Results. The results of the platings of Series A and Series B at different intervals are given in the following table. Series A was started under aerobic conditions and altered at the 30th day to anaerobic. In the case of Series B, the order was reversed. Photographs of the petri dishes were made, and are given in Plate X.

These results show more strikingly if the bacterial numbers are plotted in graphic form as in Fig. 1.

The relation of oxygen requirement to the growth of the organism is clear. The results obtained by reversing aerobic to anaerobic conditions,

Table V.

Showing the effect of aerobic and anaerobic conditions in sterile soil.
Bacteria M.P.G.

Initial inoculum 188 M.P.G.

Day	Series A		Series B	
3	Aerobic	501·0	Anaerobic	275·0
7		453·0		295·0
14		1025·0		52·0
30		444·5		15·0
(Condition reversed)				
33	Anaerobic	452·0	Aerobic	56·5
37		529·5		127·0
44		341·0		650·0
60		70·0		875·0

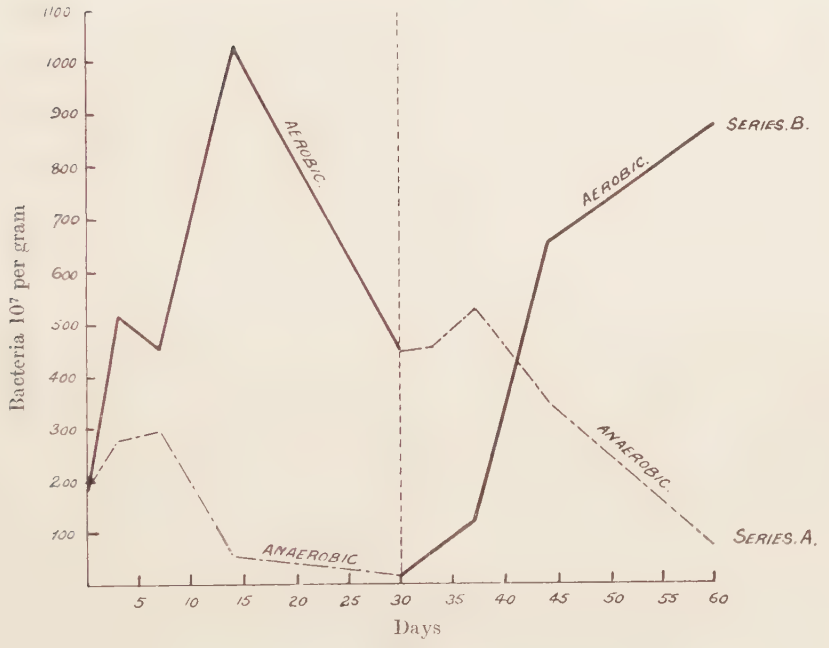


Fig. 1.

and *vice versa*, emphasise the influence the presence or absence of air exerts on the activities of the organism.

The morphological changes through which the organisms went in this experiment are shown in Figs. 2 and 3 where the percentage of each stage is plotted against the time.

Series A.

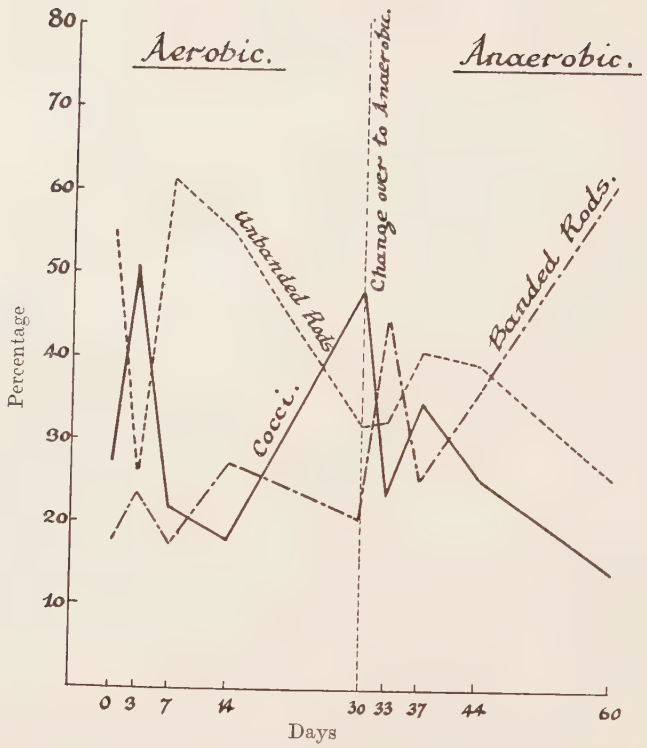


Fig. 2.

Series B.

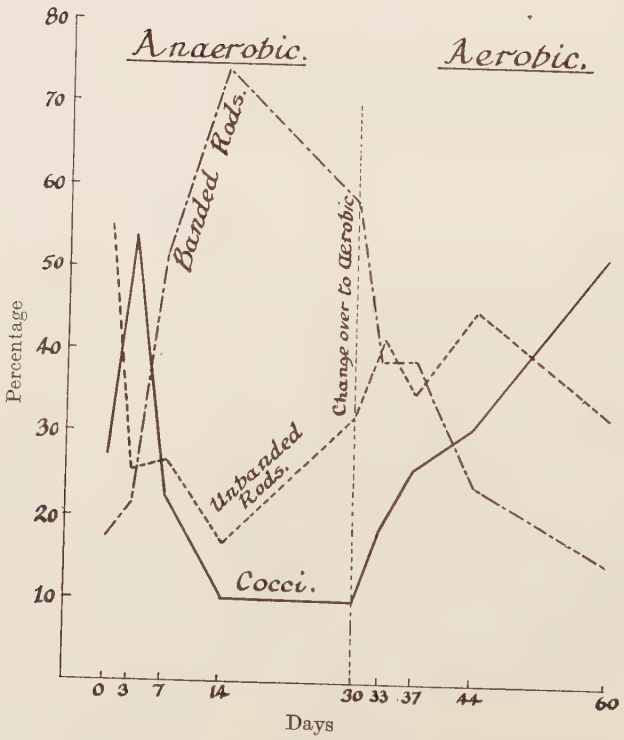


Fig. 3.

The point of interest in these two diagrams lies in the rise or fall in percentage numbers of cocci and banded rods. In the second half of the experiment, after the reversal, when the organisms had well established themselves in the soil, the cocci rise under conditions favourable for increased multiplication and fall under the more adverse anaerobic conditions. Thus it may be concluded that aeration in soil is favourable for multiplication, and that this increase in number is associated with the percentage rise in the coccoid form.

Effect of different degrees of temperature on the organism in soil.

In view of divergent results obtained by several investigators in regard to the effect of different degrees of temperature on growth of bacteria in soil, it was decided to cultivate the lucerne organism in soil at varying temperatures between 0° C. and 50° C.

Method. Ten grams of garden soil that had been passed through a 3 mm. sieve: and the moisture made up to 18·5 per cent. on air dry basis were put into each of 13 large test tubes of uniform size. The tubes were autoclaved at 15 lbs. for half an hour. The bacterial suspension was made by pouring 1 c.c. of sterile distilled water with a 14 days old culture on root extract saccharose agar. Three drops of the bacterial suspension were added to the soil in each test tube and mixed in with a sterile glass rod. The contents of one tube were used for plating immediately after inoculation. The test tubes were incubated at the following different temperatures, namely, 0°, 20°, 25°, 30°, 35° and 50° C., allotting two tubes for each grade of temperature. Soil moisture was controlled within narrow limits by weighing the tubes at intervals of three days and the loss of moisture was made up by adding sterile distilled water. The platings were made after 7 and 30 days; and slides were prepared for observing any morphological changes that may have been induced by these temperatures.

Results. The results of the platings for the two periods are given in the following table, and illustrated photographically in Plate XI.

It is evident from these results that the optimum temperature of those studied is 20° C. The freezing point temperature did not act altogether unfavourably, but the activity of the organism appears to have been depressed after 30 days' incubation. A month's incubation at 35° C. has considerably reduced the numbers. It is interesting to observe that in soil some of the organisms remained alive at 50° C. till the 7th day.

Table VI.

Showing bacteria in millions per gram of soil.

	Initial inoculum, 120·2 millions per gm. of soil	
Temperature	After 7 days' incubation	After 30 days' incubation
0° C.	221·0	120·5
20° C.	Not able to count	1092·5
25° C.	Not able to count	717·5
30° C.	174·2	179·0
35° C.	69·5	19·3
50° C.	13·0	none
	The colonies too dense	Calculated from the higher dilution

While at temperatures 0° and 35° C., the organism survives for a length of time, the fact that its optimum temperature in soil lies between 20° and 25° C. has some practical bearing. Jones (8) studying the effect of soil temperature on the development of lucerne, red clover, field beans and soy beans, found that maximum production of nodules occurred at about 24° C.

The morphological changes at 0°, 20° and 35° C. are shown from the estimate of percentage of different forms.

Table VII.

Period of incubation	Temperature	Percentage of different forms. Cocci	Rods	Banded rods	Remarks
7 days	0° C.	9·0	26·0	65·0	
	20° C.	33·7	54·7	11·6	
	35° C.	24·0	39·0	37·0	Clumps of organism
14 days	0° C.	10·7	22·0	67·0	Clumps of organism
	20° C.	15·0	38·8	46·2	
	35° C.	13·0	25·6	61·4	Clumps of organism

In the first place the increase in the percentage of banded rods appears to be associated with the extreme temperatures at which the bacterial activities are depressed. The increase in the bacterial counts is obtained from the cultures which have higher percentages of cocci and rods. It was observed that, at extreme temperatures, the organism formed into clumps around soil particles.

Effect of differing manurial treatment on the organism.

In this work soils from Hoos Field having known treatment were used in investigating the influence of manuring on the life-cycle of the organism. To ascertain its activity in such soils, quantitative estimates at intervals were made.

Method. Samples were taken from the following plots:

Plot	Treatment received since 1852
10	No manure
20	Superphosphate only
30	K, Mg, Na only
40	Complete minerals
72	Farmyard manure

One hundred grams of soil from each of the above plots were passed through a 3 mm. sieve and the moisture made up to 20 per cent. The flasks were then autoclaved.

One c.c. of the bacterial suspension made in sterile nitrogen-free mineral salts was added to each flask and well mixed with sterile rods. A month old culture grown on lucerne root extract saccharose agar was used for the inoculum.

The flasks were incubated at 20° C. and the platings were made after 7 and 14 days. One c.c. of the suspension used in inoculating the soils was plated and found to contain 109·5 millions of viable cells.

Results. The results of the platings are given below:

Table VIII.

	Bacteria millions per gm. of soil. At the start 1·095	
	After 7 days	After 14 days
Plot 10. No manure	303·0	494·0
,, 20. Superphosphate only	432·0	567·0
,, 30. K, Na, Mg only	329·5	513·0
,, 40. Complete minerals	509·0	652·0
,, 72. Farmyard manure	24·5	27·0

Thus it appears that the bacterial activity in the soil is influenced by the previous manurial treatment of the soil. In the soil from the plot that received complete minerals (superphosphate and sulphates of potash, soda and magnesia) the number of organisms on both occasions was distinctly higher than in the case of the other soils. This increase is due to the combined action of the superphosphate with the salts of K, Na and Mg; for, the soil treated with alkali salts gave a number of organisms not significantly greater than that of the unmanured plot. The low numbers obtained from the soil treated with farmyard manure are at present not understood, and further work has been taken in hand to investigate this point[1].

[1] In view of the results obtained in the experiment showing excessive depression of bacterial growth in the soil from plot 72, the experiment was repeated. The platings again gave comparatively low numbers for this soil as in the previous experiment.

The morphological variations in the soils referred to above were determined, and percentages of the different forms are given in Table IX.

Table IX.

Solids with known manurial treatment	Percentage of different forms					
	7 days			14 days		
	Cocci	Rods	Banded rods	Cocci	Rods	Banded rods
Superphosphate only	15·7	42·3	42·0	19·5	44·2	36·3
K, Na, Mg only	17·0	38·5	44·5	11·2	60·3	28·5
Complete minerals	23·6	29·1	47·3	28·0	46·9	25·1
No manure	13·4	30·9	55·7	20·9	47·9	31·2
Farmyard manure	11·5	30·5	58·0	12·0	37·0	51·0

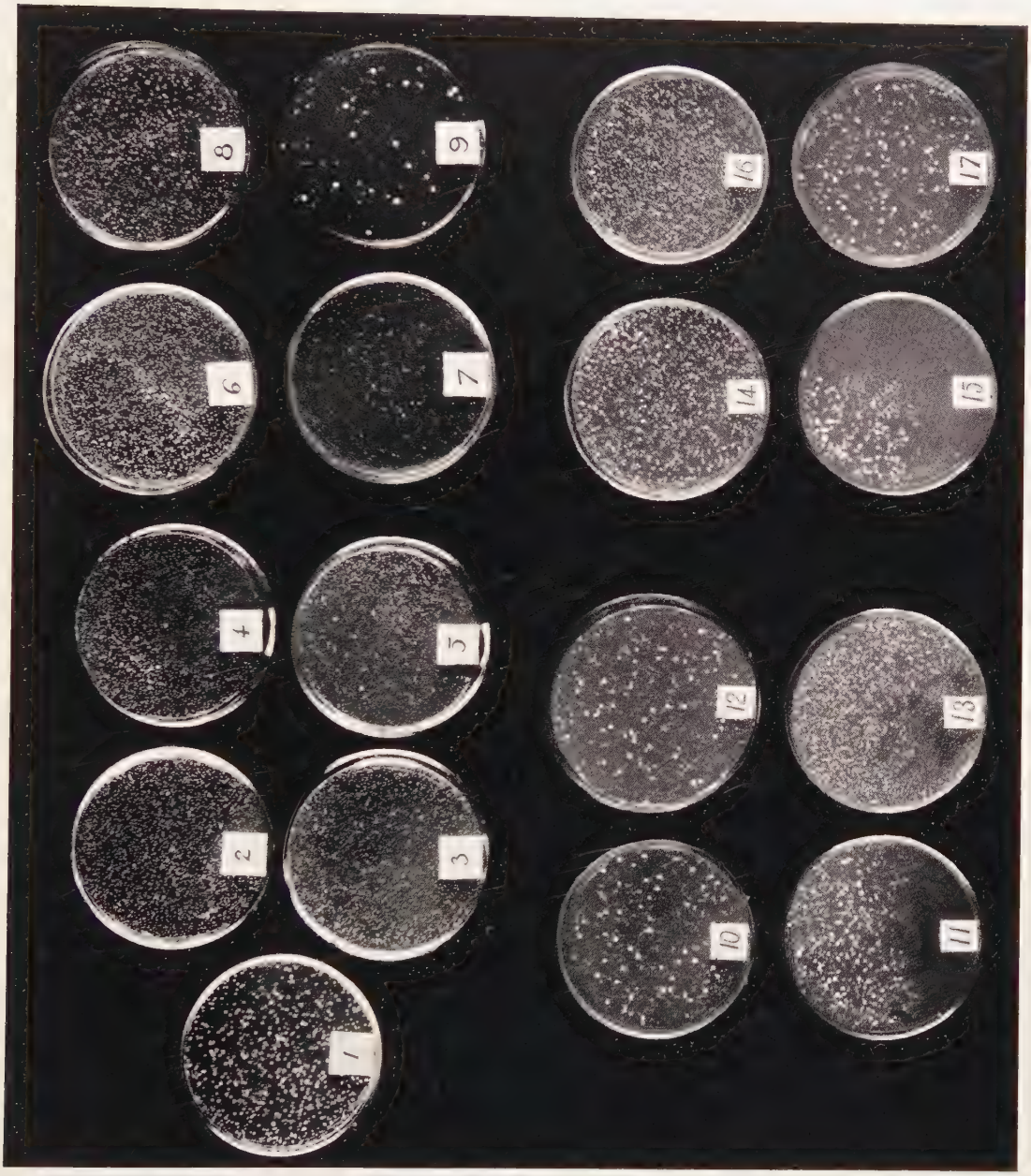
The activity of the organism, as indicated by the number per gram of soil, appears to be significantly increased in the soil manured with complete minerals, and this increase is correlated with the stage when the percentage of cocci is high. With farmyard manure, on the other hand, a high percentage of the banded rods is associated with a low bacterial count. The morphological variations as influenced by soil conditions with known manurial treatment are being further investigated.

Summary.

It is observed that on whatever media the organism is growing, whether in liquid or agar or in soil, the various stages of the life-cycle are found to occur simultaneously, but in varying proportions. The soil conditions, such as aeration, temperature and the presence of certain salts, are among the factors that determine which of the stages shall be in predominance. The relative efficiency of liquid and solid media in bringing about the predominance of a particular form was also studied.

In the main, the existence of the five stages in the life-cycle of the organism observed by Bewley and Hutchinson is confirmed; but evidence is obtained to show that under very favourable conditions the motile cocci ("swarmer") can emerge directly from the banded rod stage, developing flagella even before their emergence, and therefore missing out the non-motile ("pre-swarmer") stage.

The appearance of coccoid bodies is observed to have been accelerated in the presence of saccharose, mannite and phosphates (in agar media) and also in soil extract alone. Short rods are elongated cocci and they tend to persist in media containing the ingredients mentioned above.



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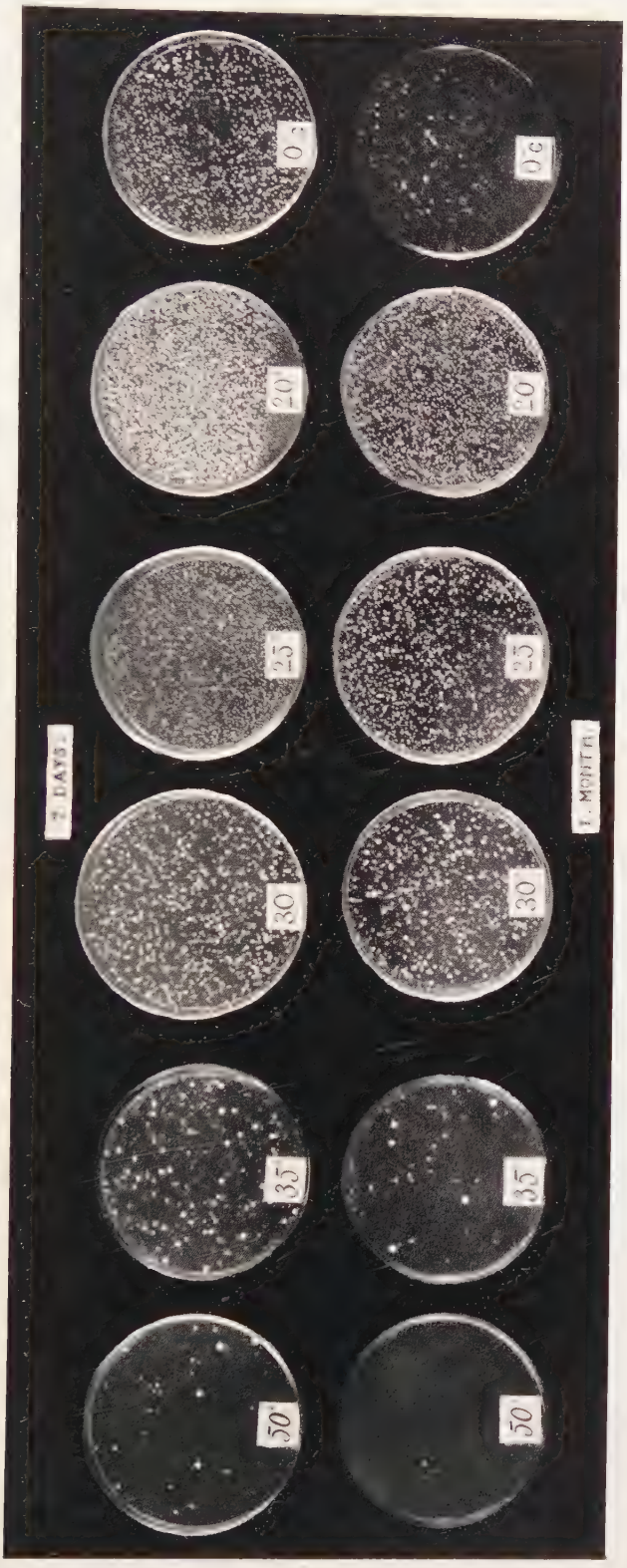

GANGULEE.—Studies on the Lucerne Nodule Organism. (360—373.)

The formation of "pre-swarmers" has been ascribed to unfavourable conditions, such as deficiency of food, or excess of calcium carbonate in the media, or inadequate aeration. A closer study of the morphological variations, however, shows that the banded rods are associated with such conditions.

Comparing the morphological variation with the number of organisms in a given time, it appears that changes in morphology are accompanied by marked alterations in the bacterial activity. The percentage increase of cocci and short rods in soil is found to be correlated with an increased total number of organisms.

The writer wishes to acknowledge his indebtedness to Mr P. H. H. Gray for giving him much valuable advice and criticism in the course of the investigation.

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EXPLANATION OF PLATES X—XI

Plate X. Effect of aerobic and anaerobic soil conditions on the Lucerne organism. 1 = initial culture. 2, 4, 6, 8 = aerobic. 3, 5, 7, 9 = anaerobic. 10, 12, 14, 16 = anaerobic converted to aerobic. 11, 13, 15, 17 = aerobic converted to anaerobic.

Plate XI. Effect of different degrees of temperature on the Lucerne organism grown in sterile soil.

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THE GROWTH OF YOUNG MICE ACCORDING TO SIZE OF LITTER

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(With 5 Text-figures.)

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I. Introductory.

The weight of an animal at birth must to a great extent be dependent upon the pre-natal nutrition, and this is especially true of a mammal like the mouse where a considerable number of foetuses are developed at the same time. The amount of nutrition which can be provided by the mother is of necessity limited, and the amount available for each individual foetus will be inversely proportional to their number. Thus in a pregnancy where only two foetuses are being developed each of these two will have a more adequate food supply than will the individuals of a litter of, say, ten. This difference in nutrition must have some reflection in the weight of the young at birth, and, as an *a priori* supposition, it might be supposed that the fewer individuals in the litter the heavier would each individual be at birth. The same argument applies with equal force to the period of lactation when the young are still dependent on the mother for sustenance, and the individuals of a large litter, probably already undersized at birth, will be further handicapped during suckling by the heavy demands on the mother. The individuals of a small litter, on the other hand, already large sized at birth might be supposed to have a better than normal growth curve during suckling owing to the plentiful supply of milk.

As a matter of fact every breeder of polytocous animals knows that this happens, and from this observation has sprung the agricultural and

laboratory practice of allowing the mother to keep only a proportion of a large litter. At the same time, however, it would seem that this question of litter size as influencing growth has not been sufficiently noticed in considering the growth curves of either mice or rats. Practically the only growth curves available for mice are those worked out by Robertson and Ray (4), and these authors merely say that there was a marked tendency for large litters to suffer from malnutrition towards the end of the suckling period and that such litters were occasionally split up and foster mothered.

The work recorded in the present paper was a study of the comparative growth of litters of different sizes, and was intended to bridge this hiatus in the literature relating to growth curves.

Material. The mice used for the experiment were from a fairly large colony which originated some four years ago from 6 pairs, and which therefore shows a fair degree of homozygosity. The animals are, of course, not of the perfection which can be produced by generations of brother and sister matings as in the case of the Wistar rats, but they are far superior to the average mice which can be obtained from dealers. The food consists of bread and milk, crushed oats and millet, and periodic vegetable food. The colony is maintained in a constant temperature of about 65° F.

The extra breeding involved by these experiments was made possible by enlargement and reconditioning of the colony carried out with the aid of a grant from the Ministry of Agriculture and Fisheries.

Methods. The females are mated with stock bucks and are removed when obviously pregnant. Litters are practically all born during the second half of the night, and the average age when first found in the morning is probably about 6 hours. In the absence of any device for trapnesting the young at birth, this means that the young have begun to suckle before the first weighing, and this first weighing therefore gives a value slightly above the actual weight at birth. A litter was weighed as a whole and the average weight of the individuals arrived at by dividing the total by the number in the litter. Twenty-two weighings of each litter were made, *i.e.* daily to the end of the 21st day.

Whatever its size the whole litter was left with the mother, though as a result of this many of the larger litters were partially eaten. Such partially eaten litters were discarded, and only completely reared litters were included in the material from which the tables were compiled. This eating of litters, especially of large ones, tends to introduce a complicating factor. Poor mothers, which would in any case rear a

litter in an unsatisfactory manner, are undoubtedly the most prone to eat some or all of the young, while the large litters which are all reared will tend to be those of the good mothers, which may be able to supply each individual of a large litter with as much nourishment as a poorer mother will be able to give to each individual of a smaller litter. Other disturbing factors of a similar nature will be considered later.

No attempt was made to select from the general stock litters of any particular size, and the relative number of each size of litter dealt with here may be considered as indicative of the normal distribution in the colony. As might be expected, the number of litters of the atypical sizes available is very few. Only one litter of 1 and two litters of 2 were produced during the time the weighings were carried on. Of the larger litters more were actually produced, but in only two and five cases respectively were litters of 10 and 9 successfully reared to 21 days old.

The curves were calculated as follows: The average weights of the individuals in each litter were summed according to the size of litter, and the totals divided by the numbers of litters of the particular size. This gave the average values for individuals of the litter size. From these values the daily increment was arrived at, and subsequently the daily percentage increment.

II. Growth Curves.

Fertility. The material consists of 66 litters comprising 407 young. The average size of litter is thus 6·2, which is remarkably similar to that of the 735 normal young (the immediate predecessors of the present material) previously reported for the colony in its present situation (3).

The slight drop is easily accounted for by the omission of a number of large litters born but not reared.

The distribution of these litters is as follows:

Table I.

Frequency distribution of litters.

Size of litter	No. of litters	No. of young
1	1	1
2	2	4
3	5	15
4	7	28
5	9	45
6	8	48
7	15	105
8	12	96
9	5	45
10	2	20
Total	66	407

For this series $\delta^2 = 4{\cdot}20$ and $\delta = 2{\cdot}05$, which is almost identical with the previous scatter values found for mice (Parkes (3)).

Weight at birth. The weight at the first weighing according to size of litter was as follows:

Table II.

Weight at birth according to litter size.

Size of litter	No. of litters	Sum of litter averages	General average
1	1	1·8	1·80
2	2	3·2	1·60
3	5	7·7	1·54
4	7	10·9	1·56
5	9	13·2	1·47
6	8	11·2	1·40
7	15	20·7	1·38
8	12	15·8	1·32
9	5	6·0	1·20
10	2	2·4	1·20
Total	66	92·9	1·41

The mean average weight at birth of the 407 mice is thus 1·41 gm., but this table also shows how the average weight at birth is greatest in small litters and sinks continuously as the scale of litter size is ascended. The solitary individual in the litter of 1 was 50 per cent. heavier than the average for the individuals of the litters of 10. Only one break occurs in the series, and this is due to the fact that the average for individuals from litters of 4 is slightly greater than that for individuals from litters of 3. In general, however, the inverse correlation between weight at birth and size of litter is well marked. This correlation is shown in Fig. 1.

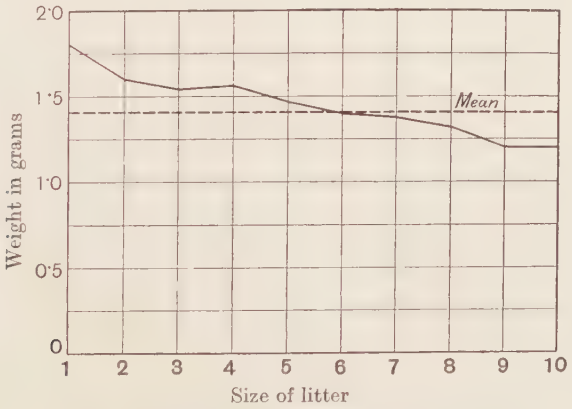

Fig. 1. Weight at birth according to litter size.

Weight at weaning. Turning now to the weight at weaning time it is found that the influence of litter size is even more pronounced. The actual figures are as follows:

Table III.

Weight at weaning according to size of litter.

Size of litter	No. of litters	Sum of litter averages	General average
1	1	16·0	16·0
2	2	23·5	11·75
3	5	44·1	8·82
4	7	57·6	8·23
5	9	69·5	7·72
6	8	55·5	6·94
7	15	98·8	6·58
8	12	71·9	5·99
9	5	25·8	5·16
10	2	8·9	4·45
Average	66	471·6	7·14

The solitary individual of the only litter of 1 was almost the size of an adult mouse at weaning, and weighed three and a half times as much as the average for litters of 10. The intermediate litter sizes grade fairly evenly in between these two extremes. The average of 7·14 for all litters falls in between the average for litters of 5 and the average for litters of 6.

The influence of litter size upon weight at birth is shown in the following diagram (Fig. 2):

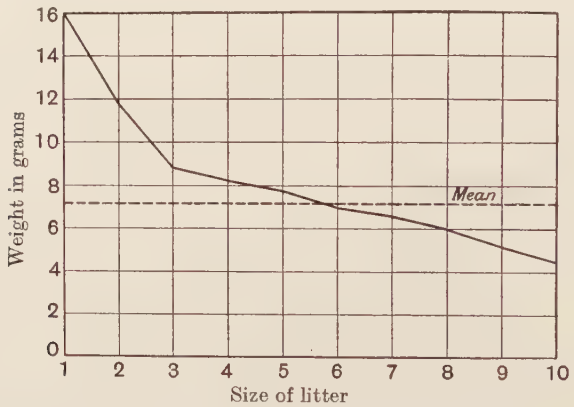

Fig. 2. Weight at weaning according to litter size.

The general average curve. Before considering the separate curves for individual sizes of litter, it will be convenient to consider the curve given by the averages of the whole 66 litters. The whole material is summarised in Table IV. In this table is given, for each size of litter, the sum of the individual litter averages at each day of age. The total for each day given in the 4th line from the bottom, divided by the total number of litters (66) gives the general average for all the individuals at each day of age (3rd line from bottom).

Table IV.

Summary of material showing average for all animals.

Size of litter	Day of observation										
	0	1	2	3	4	5	6	7	8	9	10
1 (1)	1·8	2·3	2·9	3·4	4·0	4·5	5·7	7·0	8·0	9·0	10·0
2 (2)	3·2	3·7	4·7	5·9	7·2	8·4	9·6	10·7	11·6	12·8	13·9
3 (5)	7·7	8·9	10·0	11·9	14·0	15·9	18·0	20·5	22·5	24·5	26·3
4 (7)	10·9	13·0	15·2	17·9	20·7	23·7	26·7	29·2	31·5	33·8	36·6
5 (9)	13·2	15·6	18·5	21·7	24·6	27·4	30·2	33·1	35·9	38·3	41·2
6 (8)	11·2	13·0	15·0	16·7	18·9	21·6	24·3	26·6	29·0	30·9	32·9
7 (15)	20·7	24·1	27·9	31·8	36·2	40·9	45·5	50·2	55·1	58·6	61·4
8 (12)	15·8	18·2	20·7	23·7	27·4	30·7	33·9	37·4	40·6	43·7	46·2
9 (5)	6·0	6·8	7·7	8·9	10·1	11·2	12·4	13·9	15·3	16·6	17·7
10 (2)	2·4	2·6	3·0	3·3	3·7	4·1	4·6	5·0	5·5	5·8	6·1
Whole of litters (66)	92·9	108·2	125·6	145·2	166·8	188·4	210·9	233·6	255·0	274·0	292·3
Average for all animals	1·41	1·64	1·90	2·20	2·53	2·85	3·19	3·54	3·86	4·15	4·43
Increment	—	0·23	0·26	0·30	0·33	0·32	0·34	0·35	0·32	0·29	0·28
Percentage increment	—	16·3	15·9	15·8	15·0	12·6	11·9	11·0	9·0	7·5	6·8

Size of litter	Day of observation										
	11	12	13	14	15	16	17	18	19	20	21
1 (1)	10·5	11·2	12·5	13·0	14·0	14·5	14·5	14·5	15·0	15·5	16·0
2 (2)	15·1	15·8	16·5	17·1	17·7	18·5	19·5	20·5	21·5	22·5	23·5
3 (5)	27·6	28·8	30·1	31·5	32·4	33·3	35·0	36·8	38·8	41·0	44·1
4 (7)	38·4	40·4	41·8	43·3	44·3	45·6	47·7	49·9	52·6	54·8	57·6
5 (9)	43·0	44·6	46·2	47·4	48·7	50·6	53·2	56·4	60·2	64·4	69·5
6 (8)	34·7	36·2	37·7	39·0	39·7	40·9	42·5	45·3	48·3	51·7	55·5
7 (15)	64·8	67·4	69·9	71·9	73·4	75·1	77·9	82·1	87·2	92·7	98·8
8 (12)	48·2	50·0	51·8	53·1	54·1	55·3	57·0	59·9	63·6	67·7	71·9
9 (5)	18·5	18·9	19·3	19·7	20·2	20·5	21·2	22·3	23·4	24·4	25·8
10 (2)	6·4	6·6	6·8	7·1	7·3	7·6	7·9	8·1	8·4	8·7	8·9
Whole of litters (66)	307·2	319·9	332·6	343·1	351·8	361·9	376·4	395·8	419·0	443·4	471·6
Average for all animals	4·66	4·85	5·04	5·20	5·33	5·48	5·71	6·00	6·35	6·72	7·14
Increment	0·23	0·19	0·19	0·16	0·13	0·15	0·23	0·29	0·35	0·37	0·42
Percentage increment	5·2	4·1	3·9	3·2	2·5	2·8	4·2	5·1	5·8	5·8	6·3

A consideration of this table shows that, on the average for all individuals, the rate of growth increases up to the 7th day, while after this period growth gradually falls off to the 15th day, after which time a rapid increase sets in. The average for all individuals is given in Fig. 4, which illustrates readily the period of rapid growth and the comparatively stagnant stage in between. These features are emphasised by the calculation of the daily increment given in the bottom line but one of Table IV. During the 1st day of life the increment is 0·23 gm., the amount rising steadily to 0·35 gm. by the 7th day. By the 15th day the gradual decrease in growth has reduced the daily increment to 0·13 gm., but during the ensuing period of rapid growth the increment again becomes considerable, culminating in 0·42 gm., the highest recorded, for the 21st day. (For increment curve see Fig. 3.) To be accurately assessed, however, these increments must be considered in relation to the weight of the animals showing any given increment. For this purpose the increments have been further calculated as percentages of the weights given the previous day. Thus the increment during the 1st day of life (found by comparing the weight at birth with the weight at 1 day old) is calculated as a percentage of the weight at birth, the increment during the 10th day of life as a percentage of the weight at 9 days old, and so on. These percentage increments make a very different curve from that given by the gross increments. The increase in the weight of the animal

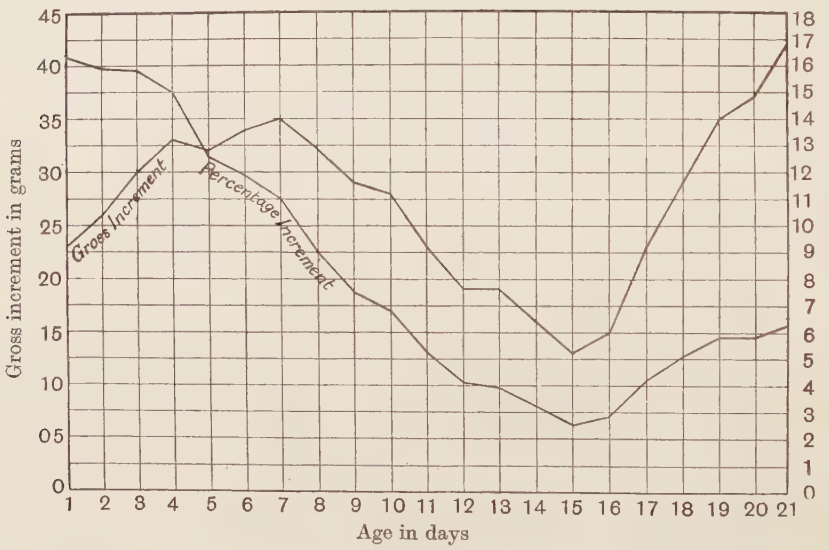

Fig. 3. Daily gross and percentage increment: average of all individuals.

obliterates the increase in the gross increment during the first 7 days; whereas the gross increment for the 1st and 7th day respectively is 0·23 and 0·35 the percentage increment is 16·3 and 11·0 respectively. This means that the percentage falls continuously from the 1st to the 15th day, it being by the latter time lowered to 2·5. From the 15th to 21st day a gradual rise is found, but the gross increment of 0·42 during the 21st day, the highest during the suckling period, represents a mere 6·3 as a percentage of the weight at the end of the 20th day. The percentage increment curve is given along with that for the gross increment in Fig. 3.

Litter of 1. The data for the solitary litter of 1 is given below in Table V.

Table V.

Data for litter of 1.

Age in days	Litter average 1	Average for litter size	Increment	Percentage increment
0	1·8	1·8	—	—
1	2·3	2·3	0·5	27·8
2	2·9	2·9	0·6	26·1
3	3·4	3·4	0·5	17·2
4	4·0	4·0	0·6	17·6
5	4·5	4·5	0·5	12·5
6	5·7	5·7	1·2	26·7
7	7·0	7·0	1·3	22·8
8	8·0	8·0	1·0	14·3
9	9·0	9·0	1·0	12·5
10	10·0	10·0	1·0	11·1
11	10·5	10·5	0·5	5·0
12	11·2	11·2	0·7	6·6
13	12·5	12·5	1·3	11·6
14	13·0	13·0	0·5	4·0
15	14·0	14·0	1·0	7·7
16	14·5	14·5	0·5	3·6
17	14·5	14·5	0·0	—
18	14·5	14·5	0·0	—
19	15·0	15·0	0·5	3·4
20	15·5	15·5	0·5	3·3
21	16·0	16·0	0·5	3·2

Since only one litter of this size was found it is hardly possible to lay much stress on the growth shown by this one animal, but at the same time it does seem possible to make two observations. In the first place, this mouse shows clearly the relatively enormous size to which a young animal will grow when there is available the whole of the natural milk supply, which for one suckling offspring is a practically

unlimited supply of nourishment. This mouse was 1·8 gm. at birth, 30 per cent. heavier than the mean. At 7 days old it weighed 7·0 gm., the mean weight at weaning time, and twice the mean of 3·54 gm. at the 7th day. At 15 days it had doubled its 7th day weight and was considerably more than twice the mean weight at 15 days. The flattening of the growth curve which normally takes place during the second week was entirely absent. During the 15–21 days period, however, it only gained 2 gm., which means that the rapid growth phase which usually closes the suckling period was entirely absent.

Litters of 2. Table VI gives the data relating to the two litters of 2 which were observed.

Table VI.

Data for litters of 2.

Age in days	Litter averages		Sum of litter averages	Average for litter size	Increment	Percentage increment
	1	2				
0	1·7	1·5	3·2	1·60	—	—
1	2·0	1·7	3·7	1·85	0·25	15·6
2	2·6	2·1	4·7	2·35	0·50	27·0
3	3·3	2·6	5·9	2·95	0·60	25·5
4	4·0	3·2	7·2	3·60	0·65	22·0
5	4·5	3·9	8·4	4·20	0·60	16·7
6	5·0	4·6	9·6	4·80	0·60	14·3
7	5·5	5·2	10·7	5·35	0·55	11·5
8	6·0	5·6	11·6	5·80	0·45	8·4
9	6·7	6·1	12·8	6·40	0·60	12·5
10	7·3	6·6	13·9	6·95	0·55	8·7
11	8·0	7·1	15·1	7·55	0·60	8·6
12	8·3	7·5	15·8	7·90	0·35	4·6
13	8·6	7·9	16·5	8·25	0·35	4·4
14	8·9	8·2	17·1	8·55	0·30	3·6
15	9·2	8·5	17·7	8·85	0·30	3·5
16	9·5	9·0	18·5	9·25	0·40	4·5
17	10·0	9·5	19·5	9·75	0·50	5·4
18	10·5	10·0	20·5	10·25	0·50	5·1
19	11·0	10·5	21·5	10·75	0·50	4·9
20	11·5	11·0	22·5	11·25	0·50	4·7
21	12·0	11·5	23·5	11·75	0·50	4·5

The average curve for these two litters shows on a lesser scale much the same kind of thing as was found for the litter of 1. The weight at birth is well above the mean (1·60 as compared with 1·41), as is the weight at weaning time (11·75 compared with 7·14). The flattening of the growth curve which normally takes place during the second week was only faintly marked. The curves for litter sizes 1 and 2 are given in Fig. 4 along with the mean curve.

Owing to the small numbers available the litters of these two sizes will be omitted from further discussion unless expressly included.

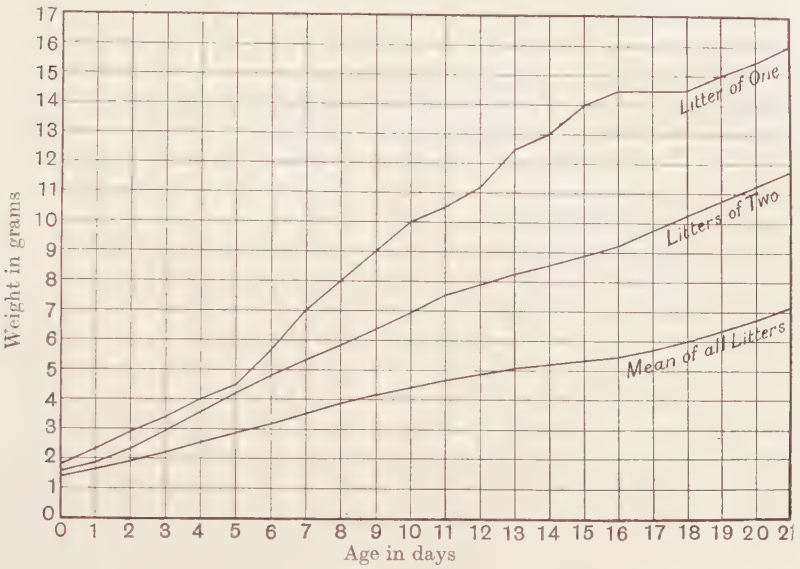

Fig. 4. Mean growth curve and curve for litter sizes 2 and 3.

Litters of 3–10. The details for the five litters of 3 available are given in Table VII.

Table VII.

Data for litters of 3.

Age in days	Litter averages 1	2	3	4	5	Sum of litter averages	Average for litter size	Increment	Percentage increment
0	1·7	1·6	1·5	1·6	1·3	7·7	1·54	—	—
1	2·0	1·9	1·7	1·7	1·6	8·9	1·78	0·24	15·6
2	2·0	2·2	1·9	1·9	2·0	10·0	2·00	0·22	12·4
3	2·7	2·4	2·2	2·2	2·4	11·9	2·38	0·38	19·0
4	3·3	2·8	2·6	2·5	2·8	14·0	2·80	0·42	17·6
5	3·7	3·1	2·9	2·9	3·3	15·9	3·18	0·38	13·6
6	4·0	3·5	3·3	3·4	3·8	18·0	3·60	0·42	13·2
7	4·5	3·9	3·7	4·0	4·4	20·5	4·10	0·50	13·9
8	4·8	4·3	4·0	4·6	4·8	22·5	4·50	0·40	9·8
9	5·3	4·5	4·2	5·2	5·3	24·5	4·90	0·40	8·9
10	5·7	4·7	4·5	5·6	5·8	26·3	5·26	0·36	7·3
11	5·7	5·0	4·7	6·0	6·2	27·6	5·52	0·26	4·9
12	5·8	5·2	4·9	6·3	6·6	28·8	5·76	0·24	4·4
13	6·3	5·3	5·1	6·4	7·0	30·1	6·02	0·26	4·5
14	6·8	5·4	5·3	6·6	7·4	31·5	6·30	0·28	4·7
15	6·7	5·7	5·5	6·8	7·7	32·4	6·48	0·18	2·9
16	6·5	6·0	5·8	7·0	8·0	33·3	6·66	0·18	2·8
17	7·0	6·3	6·1	7·4	8·2	35·0	7·00	0·34	5·1
18	7·2	6·8	6·5	7·7	8·6	36·8	7·36	0·36	5·2
19	7·5	7·3	6·9	8·1	9·0	38·8	7·76	0·40	5·4
20	7·7	7·8	7·5	8·6	9·4	41·0	8·20	0·44	5·7
21	8·2	8·5	8·2	9·2	10·0	44·1	8·82	0·62	7·5

Table VIII.

Litters of 4.

Age in days	Litter averages							Sum of litter averages	Average for litter size	Increment	Percentage increment
	1	2	3	4	5	6	7				
0	1·9	1·2	1·4	1·6	1·7	1·5	1·6	10·9	1·56	—	—
1	2·4	1·3	1·6	1·9	2·0	1·9	1·9	13·0	1·86	0·30	19·2
2	2·8	1·5	1·7	2·2	2·3	2·3	2·4	15·2	2·17	0·31	16·7
3	3·2	2·0	1·9	2·6	2·6	2·7	2·9	17·9	2·56	0·39	18·0
4	3·6	2·3	2·1	3·1	3·0	3·2	3·4	20·7	2·96	0·40	15·6
5	4·0	2·7	2·4	3·8	3·4	3·5	3·9	23·7	3·39	0·43	14·5
6	4·5	3·0	2·8	4·1	4·0	4·0	4·3	26·7	3·81	0·42	12·4
7	4·9	3·4	3·2	4·6	4·2	4·3	4·6	29·2	4·18	0·37	9·7
8	5·3	3·5	3·5	5·1	4·5	4·5	5·0	31·5	4·50	0·32	7·7
9	5·7	4·0	3·7	5·2	4·7	5·0	5·5	33·8	4·83	0·33	7·3
10	6·4	4·3	4·1	5·8	4·9	5·2	5·9	36·6	5·23	0·40	8·1
11	6·7	4·6	4·4	6·0	5·1	5·4	6·2	38·4	5·49	0·26	5·0
12	7·1	4·9	4·6	6·3	5·4	5·7	6·4	40·4	5·77	0·28	5·1
13	7·2	5·1	4·9	6·5	5·5	5·9	6·7	41·8	5·97	0·20	3·5
14	7·5	5·4	5·1	6·6	5·6	6·2	6·9	43·3	6·19	0·22	3·7
15	7·7	5·6	5·2	6·6	5·6	6·5	7·1	44·3	6·33	0·14	2·3
16	7·9	5·7	5·4	6·5	6·0	6·8	7·3	45·6	6·52	0·19	3·0
17	8·3	6·0	5·6	6·9	6·2	7·1	7·6	47·7	6·82	0·30	4·6
18	8·6	6·2	6·0	7·3	6·6	7·4	7·8	49·9	7·13	0·31	4·5
19	9·0	7·0	6·5	7·7	6·8	7·7	7·9	52·6	7·51	0·38	5·3
20	9·4	7·2	6·9	8·1	7·1	7·9	8·2	54·8	7·83	0·32	4·3
21	9·7	7·6	7·2	8·6	7·9	8·1	8·5	57·6	8·23	0·40	5·1

Table IX.

Litters of 5.

Age in days	Litter averages									Sum of litter averages	Average for litter size	Increment	Percent age increment
	1	2	3	4	5	6	7	8	9				
0	1·4	1·4	1·5	1·6	1·3	1·6	1·5	1·5	1·4	13·2	1·47	—	—
1	1·7	1·5	1·9	1·8	1·7	1·8	1·8	1·7	1·7	15·6	1·73	0·26	17·7
2	2·0	1·8	2·6	2·0	1·8	2·1	2·2	2·0	2·0	18·5	2·06	0·33	19·1
3	2·4	2·2	3·0	2·3	2·1	2·4	2·6	2·4	2·3	21·7	2·41	0·35	17·0
4	2·8	2·4	3·4	2·6	2·4	2·7	2·9	2·8	2·6	24·6	2·73	0·32	13·3
5	3·2	2·7	3·8	3·0	2·7	3·0	3·1	3·1	2·8	27·4	3·05	0·32	11·7
6	3·5	3·0	4·2	3·4	3·0	3·3	3·4	3·4	3·0	30·2	3·35	0·30	9·8
7	3·9	3·2	4·6	3·8	3·3	3·6	3·6	3·8	3·3	33·1	3·68	0·33	9·8
8	4·2	3·5	4·9	4·2	3·6	3·9	3·9	4·1	3·6	35·9	3·99	0·31	8·4
9	4·4	3·7	5·2	4·5	3·8	4·2	4·2	4·4	3·9	38·3	4·26	0·27	6·8
10	4·8	4·0	5·8	4·7	4·1	4·5	4·4	4·7	4·2	41·2	4·58	0·34	8·0
11	4·9	4·3	6·0	4·9	4·4	4·7	4·6	4·8	4·4	43·0	4·78	0·18	3·9
12	5·1	4·7	6·0	5·1	4·7	4·9	4·7	4·9	4·5	44·6	4·96	0·18	3·8
13	5·3	4·8	6·4	5·2	5·0	5·1	4·8	5·0	4·6	46·2	5·14	0·18	3·6
14	5·5	5·0	6·5	5·3	5·0	5·3	5·0	5·1	4·7	47·4	5·27	0·13	2·5
15	5·6	5·2	6·6	5·3	5·1	5·5	5·2	5·4	4·8	48·7	5·41	0·14	2·7
16	5·8	5·3	7·0	5·4	5·1	5·7	5·4	5·8	5·1	50·6	5·62	0·21	3·9
17	6·3	5·4	7·4	5·6	5·2	6·0	5·6	6·3	5·4	53·2	5·92	0·30	5·3
18	6·7	5·8	7·8	5·9	5·4	6·3	6·0	6·8	5·7	56·4	6·27	0·35	5·9
19	7·2	6·3	8·2	6·3	5·8	6·6	6·5	7·3	6·0	60·2	6·67	0·40	6·4
20	7·5	6·8	8·6	6·8	6·4	7·0	7·0	7·9	6·4	64·4	7·16	0·49	7·3
21	7·8	7·3	9·1	7·5	7·0	7·6	7·6	8·6	7·0	69·5	7·72	0·56	7·8

Table X.

Litters of 6.

Age in days	Litter averages 1	2	3	4	5	6	7	8	Sum of litter averages	Average for litter size	Increment	Percentage increment
0	1·4	1·3	1·3	1·3	1·5	1·4	1·6	1·4	11·2	1·40	—	—
1	1·6	1·4	1·6	1·6	1·8	1·6	1·8	1·6	13·0	1·62	0·22	15·7
2	1·8	1·6	2·0	1·9	2·1	1·8	2·0	1·8	15·0	1·88	0·26	16·1
3	2·1	1·8	2·3	2·1	2·3	1·9	2·3	1·9	16·7	2·09	0·21	11·2
4	2·4	2·1	2·7	2·4	2·5	2·1	2·6	2·1	18·9	2·37	0·28	13·4
5	2·8	2·4	3·1	2·7	3·1	2·3	2·9	2·3	21·6	2·70	0·33	13·6
6	3·1	2·7	3·4	3·1	3·6	2·6	3·2	2·6	24·3	3·04	0·34	12·6
7	3·4	3·1	3·7	3·3	3·9	2·8	3·6	2·8	26·6	3·33	0·23	7·6
8	3·6	3·5	4·0	3·6	4·2	3·2	3·9	3·0	29·0	3·63	0·36	11·0
9	3·8	3·8	4·3	3·8	4·5	3·4	4·1	3·2	30·9	3·86	0·23	6·3
10	4·0	4·1	4·6	3·9	4·8	3·6	4·4	3·5	32·9	4·11	0·25	6·5
11	4·1	4·3	4·8	4·0	5·4	3·8	4·7	3·6	34·7	4·34	0·23	5·6
12	4·3	4·4	4·9	4·1	5·7	4·1	5·0	3·7	36·2	4·53	0·19	4·4
13	4·4	4·6	5·2	4·2	5·9	4·5	5·2	3·7	37·7	4·72	0·19	4·2
14	4·5	4·9	5·3	4·3	6·3	4·5	5·4	3·8	39·0	4·87	0·15	3·2
15	4·6	4·8	5·5	4·3	6·4	4·6	5·6	3·9	39·7	4·97	0·10	2·1
16	4·7	4·7	5·7	4·4	6·8	4·8	5·8	4·0	40·9	5·11	0·14	2·8
17	4·9	4·7	5·7	4·7	7·2	5·2	6·0	4·1	42·5	5·32	0·20	3·8
18	5·4	5·0	6·1	5·1	7·5	5·5	6·4	4·3	45·3	5·67	0·36	6·8
19	5·9	5·3	6·4	5·5	7·7	6·1	6·8	6·6	48·3	6·04	0·37	6·3
20	6·2	5·9	6·8	5·9	8·1	6·4	7·3	5·1	51·7	6·47	0·43	6·9
21	6·5	6·3	7·3	6·3	8·3	7·0	8·0	5·8	55·5	6·94	0·47	7·3

Table XI.

Litters of 7.

Age in days	Litter averages 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Sum of litter av.	Av. for litter size	Av. increment	Percentage increment
0	1·4	1·3	1·4	1·5	1·7	1·4	1·5	1·2	1·3	1·1	1·2	1·3	1·6	1·4	1·4	20·7	1·38	—	—
1	1·6	1·5	1·6	1·7	1·9	1·7	1·9	1·4	1·6	1·3	1·4	1·5	1·8	1·6	1·6	24·1	1·61	0·16	11·6
2	1·9	1·8	1·9	1·9	2·1	2·0	2·1	1·6	1·9	1·5	1·6	1·8	2·1	1·9	1·8	27·9	1·86	0·32	20·8
3	2·2	2·1	2·2	2·2	2·6	2·3	2·3	1·8	2·1	1·7	1·7	2·0	2·4	2·1	2·1	31·8	2·12	0·26	14·0
4	2·6	2·5	2·5	2·5	2·9	2·7	3·7	1·9	2·4	2·0	1·8	2·3	2·7	2·3	2·4	36·2	2·41	0·29	13·7
5	3·0	2·8	2·8	2·8	3·3	3·2	3·2	2·1	2·7	2·3	1·9	2·5	3·0	2·6	2·7	40·9	2·73	0·32	13·3
6	3·3	3·3	3·2	3·2	3·7	3·5	3·5	2·3	2·9	2·7	2·1	2·7	3·3	2·9	3·0	45·5	3·03	0·30	11·0
7	3·6	3·5	3·5	3·4	4·1	3·9	3·9	2·5	3·1	3·3	2·3	2·9	3·7	3·2	3·3	50·2	3·35	0·32	10·6
8	3·9	3·9	3·8	3·6	4·7	4·2	4·6	2·7	3·6	3·6	2·5	3·1	3·9	3·4	3·6	55·1	3·67	0·32	9·6
9	4·2	4·0	4·0	3·9	5·1	4·4	4·9	2·8	4·0	3·8	2·8	3·2	4·1	3·6	3·8	58·6	3·91	0·24	6·5
10	4·3	4·2	4·1	4·2	5·3	4·7	5·1	2·9	3·9	4·0	3·2	3·5	4·2	3·8	4·0	61·4	4·09	0·18	4·6
11	4·4	4·3	4·4	4·3	5·7	5·0	5·3	3·1	4·2	4·3	3·6	3·7	4·3	4·0	4·2	64·8	4·32	0·25	6·1
12	4·6	4·6	4·6	4·4	5·9	5·1	5·6	3·2	4·4	4·5	3·8	3·9	4·4	4·1	4·3	67·4	4·49	0·15	3·5
13	4·7	4·7	4·8	4·6	6·0	5·3	6·0	3·4	4·6	4·7	4·0	4·0	4·5	4·3	4·3	69·9	4·66	0·17	3·8
14	4·8	4·9	4·9	4·8	6·2	5·1	6·3	3·5	4·8	4·9	4·2	4·1	4·6	4·4	4·4	71·9	4·80	0·14	3·0
15	4·8	5·0	5·0	4·9	6·3	5·5	6·3	3·6	5·0	5·0	4·3	4·2	4·7	4·4	4·4	73·4	4·89	0·09	1·9
16	4·9	5·2	5·1	4·9	6·3	5·6	6·4	3·8	5·2	5·1	4·4	4·4	4·8	4·5	4·5	75·1	5·05	0·16	3·3
17	5·2	5·3	5·2	5·1	6·9	5·7	6·5	4·0	5·3	5·2	4·6	4·6	5·0	4·6	4·7	77·9	5·19	0·14	2·8
18	6·0	5·7	5·6	5·4	7·1	5·9	6·6	4·2	5·5	5·3	4·8	4·8	5·4	4·8	5·0	82·1	5·47	0·28	5·4
19	6·2	6·5	6·0	5·9	7·4	6·2	6·7	4·4	5·9	5·5	5·1	5·1	5·8	5·2	5·3	87·2	5·81	0·34	6·2
20	6·3	6·9	6·4	6·0	7·8	6·5	7·3	4·7	6·1	5·9	5·4	5·5	6·4	5·7	5·8	92·7	6·18	0·37	6·4
21	6·3	7·1	7·0	6·3	8·3	6·9	7·8	5·0	6·3	6·2	5·9	6·2	6·8	6·3	6·4	98·8	6·58	0·30	4·9

Table XII.

Litters of 8.

Age in days	Litter averages 1	2	3	4	5	6	7	8	9	10	11	12	Sum of litter averages	Average for litter size	Increment	Percentage increment
0	1·3	1·2	1·5	1·2	1·4	1·5	1·3	1·3	1·4	1·1	1·3	1·3	15·8	1·32	—	—
1	1·6	1·3	1·6	1·4	1·6	1·9	1·4	1·5	1·7	1·2	1·5	1·5	18·2	1·52	0·20	15·2
2	1·8	1·5	1·7	1·8	1·7	2·1	1·6	1·8	1·9	1·4	1·7	1·7	20·7	1·73	0·21	13·8
3	2·2	1·7	2·1	2·1	1·8	2·3	1·8	2·1	2·3	1·5	1·9	1·9	23·7	1·97	0·24	13·8
4	2·6	2·0	2·4	2·4	2·2	2·6	2·0	2·4	2·6	1·7	2·3	2·2	27·4	2·28	0·31	15·7
5	2·9	2·3	2·6	2·7	2·4	2·8	2·2	2·8	3·1	1·9	2·5	2·5	30·7	2·56	0·28	12·4
6	3·3	2·5	2·9	3·0	2·7	3·1	2·4	3·2	3·2	2·2	2·7	2·7	33·9	2·83	0·27	10·4
7	3·7	2·8	3·2	3·3	2·9	3·4	2·6	3·5	3·5	2·6	3·0	2·9	37·4	3·12	0·29	10·2
8	4·1	3·0	3·7	3·6	3·2	3·6	2·8	3·8	3·7	2·8	3·2	3·1	40·6	3·38	0·26	8·3
9	4·5	3·2	4·0	3·8	3·5	4·0	2·9	4·2	3·9	3·0	3·4	3·3	43·7	3·64	0·26	7·7
10	4·7	3·6	4·4	4·0	3·6	4·1	3·1	4·4	4·1	3·2	3·5	3·5	46·2	3·85	0·21	5·8
11	5·0	3·9	4·5	4·1	3·7	4·2	3·3	4·5	4·3	3·4	3·6	3·7	48·2	4·02	0·17	4·4
12	5·2	4·0	4·7	4·2	3·8	4·4	3·5	4·6	4·5	3·5	3·7	3·9	50·0	4·16	0·14	3·5
13	5·3	4·1	4·8	4·4	4·0	4·7	3·6	4·7	4·7	3·6	3·8	4·1	51·8	4·32	0·16	3·8
14	5·4	4·1	4·9	4·5	4·1	4·9	3·7	4·7	4·8	3·9	4·0	4·1	53·1	4·42	0·11	2·6
15	5·5	4·2	4·9	4·6	4·1	5·1	3·9	4·8	4·8	3·9	4·1	4·2	54·1	4·51	0·08	1·8
16	5·6	4·2	5·0	4·7	4·3	5·4	4·1	4·8	4·9	4·0	4·1	4·2	55·3	4·61	0·10	2·2
17	5·7	4·2	5·1	4·8	4·4	5·7	4·4	4·9	5·1	4·2	4·2	4·3	57·0	4·75	0·14	3·0
18	6·0	4·3	5·4	4·9	4·6	6·0	4·7	5·2	5·4	4·4	4·5	4·5	59·9	4·99	0·24	5·1
19	6·5	4·5	5·6	5·1	5·0	6·3	5·2	5·5	5·8	4·6	4·8	4·7	63·6	5·30	0·31	6·2
20	7·0	5·0	5·9	5·4	5·3	6·6	5·4	5·8	6·3	4·9	5·1	5·0	67·7	5·64	0·34	6·4
21	7·3	5·5	6·2	5·6	5·8	6·9	5·7	6·1	6·9	5·2	5·4	5·3	71·9	5·99	0·35	6·2

Table XIII.

Litters of 9.

Age in days	Litter averages 1	2	3	4	5	Sum of litter averages	Average for litter size	Increment	Percentage increment
0	1·4	1·0	1·1	1·2	1·3	6·0	1·20	—	—
1	1·6	1·3	1·2	1·3	1·4	6·8	1·36	0·16	13·3
2	1·9	1·5	1·3	1·4	1·6	7·7	1·54	0·18	13·2
3	2·4	1·8	1·4	1·6	1·7	8·9	1·78	0·24	15·6
4	2·8	2·0	1·5	1·9	1·9	10·1	2·02	0·24	13·5
5	3·1	2·2	1·6	2·2	2·1	11·2	2·24	0·22	10·9
6	3·2	2·5	2·0	2·3	2·4	12·4	2·48	0·24	10·7
7	3·5	2·8	2·4	2·5	2·7	13·9	2·78	0·30	12·1
8	3·8	3·0	2·8	2·7	3·0	15·3	3·06	0·28	10·1
9	3·9	3·2	3·2	2·9	3·4	16·6	3·32	0·26	8·6
10	4·2	3·4	3·5	3·0	3·6	17·7	3·54	0·22	6·6
11	4·4	3·6	3·7	3·1	3·7	18·5	3·70	0·16	4·5
12	4·5	3·6	3·8	3·2	3·8	18·9	3·78	0·08	2·2
13	4·6	3·7	3·8	3·3	3·9	19·3	3·86	0·08	2·1
14	4·6	3·8	4·0	3·4	3·9	19·7	3·94	0·08	2·0
15	4·7	3·9	4·1	3·5	4·0	20·2	4·04	0·10	2·5
16	4·7	3·9	4·2	3·6	4·1	20·5	4·10	0·06	1·5
17	4·9	4·1	4·3	3·7	4·2	21·2	4·24	0·14	3·4
18	5·2	4·4	4·6	3·8	4·3	22·3	4·46	0·22	5·2
19	5·6	4·6	4·8	3·9	4·5	23·4	4·68	0·22	4·9
20	5·7	4·9	5·1	4·0	4·7	24·4	4·88	0·20	4·3
21	5·8	5·2	5·7	4·1	5·0	25·8	5·16	0·28	5·8

Table XIV.

Litters of 10.

Age in days	Litter averages 1	Litter averages 2	Sum of litter averages	Average for litter size	Increment	Percentage increment
0	1·2	1·2	2·4	1·20	—	—
1	1·3	1·3	2·6	1·30	0·10	8·3
2	1·5	1·5	3·0	1·50	0·20	15·4
3	1·6	1·7	3·3	1·65	0·15	10·0
4	1·8	1·9	3·7	1·85	0·20	12·1
5	2·0	2·1	4·1	2·05	0·20	10·8
6	2·2	2·4	4·6	2·30	0·25	12·1
7	2·4	2·6	5·0	2·50	0·20	8·7
8	2·6	2·9	5·5	2·75	0·25	10·0
9	2·7	3·1	5·8	2·90	0·15	5·5
10	2·8	3·3	6·1	3·05	0·15	5·2
11	2·9	3·5	6·4	3·20	0·15	4·9
12	3·0	3·6	6·6	3·30	0·10	3·1
13	3·2	3·6	6·8	3·40	0·10	3·0
14	3·5	3·6	7·1	3·55	0·15	4·4
15	3·7	3·6	7·3	3·65	0·10	2·8
16	3·9	3·7	7·6	3·80	0·15	4·1
17	4·1	3·8	7·9	3·95	0·15	3·9
18	4·3	3·8	8·1	4·05	0·10	2·5
19	4·5	3·9	8·4	4·20	0·15	3·7
20	4·7	4·0	8·7	4·35	0·15	3·6
21	4·8	4·1	8·9	4·45	0·10	2·3

The litters of this size give a curve which is essentially of the same nature as the mean curve, the difference being one of degree only. The two phases of growth are observable during the first and third weeks, and the temporary slackening off in growth during the second week is also shown. The gradient throughout, however, is greater than in the case of the mean curve, and greater than the curve for litter sizes 4–10.

Since the curves for all the remaining sizes of litter (4–10) are of precisely similar nature, they may be considered together.

From these tables, together with the weight-age-size of litter surface shown in Fig. 5 the following observations may be made:

(*a*) For sizes of litter of 3–10 the type of curve is identical with the mean curve for all the animals examined, *i.e.* an initial period of rapid growth, followed by a temporary grading down in the growth which is in turn succeeded by a final period of heavy growth. The mean curve for all individuals falls between the curve for litter sizes 5 and 6; litter sizes 3, 4, 5 show a higher gradient of growth than the mean, while litters of 6, 7, 8, 9, 10 show a lower gradient. In litters of 10 the growth phases are noticeably depreciated.

(*b*) The gross increment is usually greatest during the last two or three days of suckling, and least during the phase of slow growth during the second week. The increment relative to size (percentage increment)

is, however, far greater in the initial burst of growth than during the final phase.

(*c*) As would be expected from the growth curves, the daily gross increment sinks in the larger sizes of litters. In litters of 3, for example, 0·62 gm. average increase was observed during the 21st day, while in

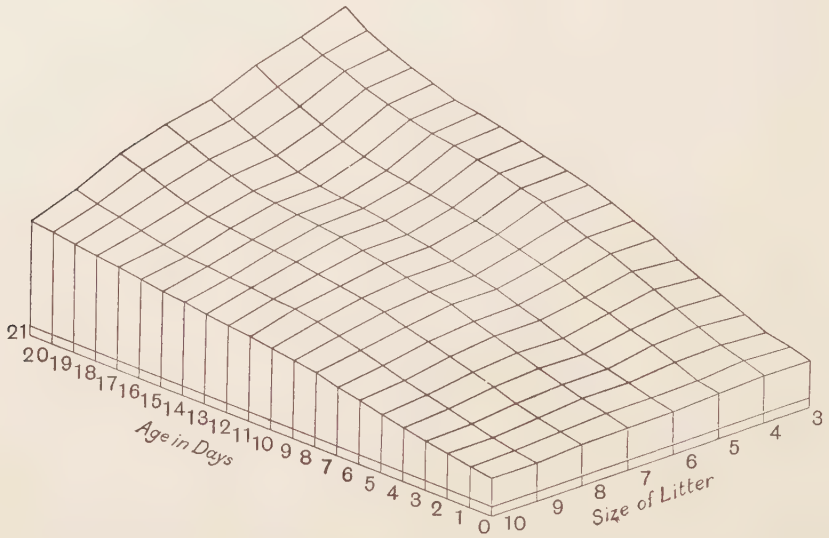

Fig. 5. Weight surface according to size of litter and age.

litters of 9 the highest daily increment observed during the second growth phase was 0·28 gm. on the 21st day; in litters of 10 it was 0·15 gm. on the 20th day. During the first growth phase 0·5 gm. increment was observed for litters of 3, while the highest during this time was 0·3 on the 7th day for litters of 9 and 0·25 on the 6th day for litters of 10.

The following table summarises the material from this point of view:

Table XV.

Comparison of gross increment according to age and litter size.

Size of litter	Highest of first growth phase (and day)	Gross increment lowest during ebb phase (and day)	Highest of second growth phase (and day)
3	0·5 (7th)	0·18 (15–16th)	0·62 (21st)
4	0·43 (5th)	0·14 (15th)	0·4 (21st)
5	0·33 (7th)	0·13 (14th)	0·56 (21st)
6	0·34 (6th)	0·1 (15th)	0·47 (21st)
7	0·32 (5–6–8th)	0·09 (15th)	0·37 (20th)
8	0·31 (4th)	0·08 (15th)	0·35 (21st)
9	0·3 (7th)	0·06 (16th)	0·28 (21st)

(Litters of 10 omitted on account of small numbers.)

This table shows clearly the difference between the gross increment at the points of most and least growth. Considerable variation is also found in the different sizes of litter. The greatest increment during the first growth period varies between 0·5 and 0·3 gm. and during the ebb period the variation is between 1·8 and 0·06 gm.; and during the last stage between 0·62 and 0·28 gm.

The actual increase in weight, therefore, declines greatly with increasing litter size.

(*d*) The percentage increment, however, though showing very wide variation in the different growth phases, shows far less variation from one litter size to another than does the gross increment.

In litters of 3 the highest percentage increment is 19·0 gm. during the 3rd day and the lowest 2·8 on the 16th day, while from this lowest ebb the percentage rises again to 7·5 on the 21st day. For comparative purposes a summary table may be made of these data for the different litter sizes.

Table XVI.

Comparison of percentage increments according to age and litter size.

Size of litter	Highest of first growth phase (and day)	Lowest during ebb phase (and day)	Highest of second growth phase (and day)
3	19·0 (3rd)	2·8 (16th)	7·5 (21st)
4	19·2 (1st)	2·3 (15th)	5·1 (21st)
5	19·1 (2nd)	2·5 (14th)	7·8 (21st)
6	16·1 (2nd)	2·1 (15th)	7·3 (21st)
7	20·8 (2nd)	1·9 (15th)	6·4 (20th)
8	15·7 (4th)	1·8 (15th)	6·4 (20th)
9	15·6 (3rd)	1·5 (16th)	5·8 (21st)

(Litters of 10 omitted on account of small numbers.)

This table shows clearly the fact mentioned above, *i.e.* that the percentage increment varies greatly during the successive growth phases of the suckling period, but that far less variation according to litter size is found in the percentage increment than in the gross increment. The most striking thing is the regularity with which the lowest point is reached at about the 15th day. The highest point of the first growth phase is reached within 4 days of birth, and the actual percentages only vary from 20·8 to 15·6 in the different litter sizes. The highest point of the second growth phase is attained right at the end of the suckling period, the variation according to litter sizes being only between 5·1 and 7·5 per cent.

III. Discussion.

Comparison with Robertson and Ray's results.

In the first place the mean growth curve for the whole of the observations may be compared with Robertson and Ray's mean curve. These authors found that the average weight at birth in their mice was 1·23 gm., a figure which is noticeably less than that of 1·41 gm. which was found to be the mean weight at birth in my own observations. Robertson and Ray, however, do not say how soon after birth the litters were first weighed (though since litters are mostly born during the night, first weighing could scarcely have been immediately after) or, a more important factor, whether or not the young were "trapnested" or otherwise prevented from suckling until after the first weighing. At 7 days old their material averaged 3·31 gm. which again is below the 3·54 gm. averaged by the mice reported in this paper. Up to this point both males and females were found by Robertson and Ray to weigh the same, but by 11 days old the curves are diverging slightly. From the point of view of my own observations the question of sex difference in growth was irrelevant, and, as mentioned above, was not entered into, but since the 407 mice dealt with consisted of 207 males and 200 females it must be supposed that the average weights of the two cases diverge equally (if at all) on each side of their mean, and that average between Robertson and Ray's figures for males and females may legitimately be compared with my mean figure. At 11 days old their figures show the males weighing 4·67 gm. and the females 4·58 gm., giving an average of 4·625 gm. as compared with 4·66 gm. for my material at this age. At 14 days old their figure is 5·02 gm. as against that of 5·20 gm. for my material, while at 17 days there is again a difference, the figures being 5·59 gm. and 5·71 gm. respectively. The next figure given by the two authors is for 23 days old mice, but by interpolating it appears that at 21 days (the latest at which my stock was weighed) this material would average about 7·4 gm., which is somewhat above my figure of 7·14 gm. Since, however, the present paper makes it clear that the size of litter has a very considerable influence on the growth of the young, and more especially since Robertson and Ray divided up some of their large litters, it is apparent that another factor, *i.e.* relative average size of litter, must be taken into consideration in comparing the two sets of results. The average litter size of all Robertson and Ray's material was 5·15, whereas the average in the 407 mice under discussion was 6·20, so that if for no other reason than this it might be expected that their material

would show a better growth curve than my own. The fact that mine has a better growth curve till the 17th day is curious. Since their average litter size of 5·15 approximates most closely to 5, it is instructive to compare the growth shown by their whole material with my data for litters of 5. This is done in the following summary table:

Table XVII.

Comparison of mean weights and weights for litters of 5 with Robertson and Ray's figures.

Age in days	Robertson and Ray	Present material	
		Mean	Litters of 5
0	1·23	1·41	1·47
7	3·31	3·54	3·68
11	4·62	4·66	4·78
14	5·02	5·20	5·27
17	5·59	5·71	5·92
21	7·4	7·14	7·72
23	8·88	—	—

Robertson and Ray's mean weights are therefore less at all stages than those for litters of 5 of the present material, and less at all stages except the last than my mean weights, in spite of the larger average size of litter.

At the same time, however, the general type of curve is similar. Both my curve and that of Robertson and Ray show the two phases of growth which are found in the first 3 weeks of life. In my case the latest period of reduced growth is more pronounced, but the latest period is evident in both.

The cause of this flattening of the growth curve is not quite apparent —it may be due to some inherent property of the suckling animals themselves or it may be due to vicissitudes of nutrition. In the case of my material I incline to the latter view. The second period of growth does not really become pronounced till the 16th day, at which time the young are just beginning to eat small amounts of food for themselves. The mean curve therefore seems to be best interpreted as follows: 0–9 days—a period of rapid growth, 10–15 days—a period of progressively reduced growth when the nutrition of the young becomes progressively inadequate, 16–21 days—when the growth again becomes rapid as the young learn to eat for themselves. This explanation becomes very plausible when it is considered that the smaller the litter the less marked is the period of reduced growth, suggesting that in cases where the drain on the

mother is less acute, the young are less affected at the time when the drain on the mother is at its greatest, *i.e.* at the time when the young are at the greatest stage of development while still totally dependent on the mother, just before they begin to eat. In the case of the litter of 1 the period of reduced growth is entirely absent.

The growth according to litter size.

The question of the growth according to litter size may now be discussed and in the first place it is necessary to discuss possible disturbing factors in the growth according to litter size.

(1) Other things being equal the females in the best conditions will have large litters, while females in poor condition will have small litters. The good females may however be equally capable of nursing a large litter as a poor female is of nursing a small litter, and in such cases differential nutrition will not obtain to any pronounced extent. This factor will tend to equalise the growth curves of small and large litters.

(2) Since young and old mothers tend to produce less milk than females in their prime, the age of the mother will also be a factor in the growth of young. At the same time, however, young and old females tend to have smaller litters so that these complications will compensate for each other.

(3) Since male young are heavier than female young, litters consisting mostly of males might grow faster than litters tending towards unisexuality in the other direction, but this factor could not upset the curves to any great extent and in any case it is nullified by two other facts, (*a*) that the sexes occur in almost equal numbers, and (*b*) that no correlation between litter size and sex ratio is observable.

(4) A more serious complication is the possibility of stillbirths, which are usually eaten immediately on birth, reducing the size of the litter with the result that young of a size characteristic of a larger litter are left to represent a smaller litter size. Stillbirths, however, do not appear to be very common in mice.

It is clear therefore, that to get absolute uniformity in these variables it would be necessary to have larger series of homozygous females of the same age, having had the same number of litters previously, in the same physical condition, of the same weight and producing the same proportion of stillbirths; an ideal which is beyond practical attainment. In spite, however, of the existence of this large number of variables, which cannot be completely controlled, it seems improbable that, where large numbers of young are dealt with, these factors could

combine to give the result in question, *i.e.* an increased proportion between litter size and growth, unless an actual tendency towards this correlation existed.

The chief of the actual conclusions which require consideration are:

(*a*) That during the 3 weeks' suckling period the mean growth for the whole material examined shows two main phases; *i.e.* during the first week, and during the third week.

(*b*) This general type of growth curve is followed by all the sizes of litters to a greater or less degree, but most accurately in the cases of litter sizes 3–8 inclusive. Larger litters (8–10) do not show the last phase of growth to any extent, while in the very small litters which show very rapid growth the last growth phase is also relatively unmarked. For all litters the intensity of the growth is almost strictly inversely proportional to the size.

In putting forward an explanation of the two phases of growth found in the mean curve, it is possible to start from either of two standpoints: firstly, that the irregular growth curve is an inherent attribute of the young, or secondly, that vicissitudes of nutrition account for the two distinct phases. The fact, however, that no intermediate period of retarded growth is found where the nutrition is constantly plentiful (as in the case of the litter of 1) suggests that the former of these two hypotheses is invalid. The latter one, however, seems to afford an explanation of all the results obtained. The first phase of rapid growth would appear to be explained by the supposition that during this early stage, when the young are small, the mother is able to supply them with all the nutriment they can deal with. When, however, the young become larger the demands on the mother are probably not entirely satisfied, and this would lead to the flattening of the curve which is noticed during the second week. At about the 15th day, however, the young first begin to nibble food for themselves, and this would account for the spurt of growth which is found in the last week.

The difference in the nutrition available undoubtedly accounts for the inverse proportion between litter size and growth, and this would lead one to expect that all sizes of litter would show a rapid growth phase when the young begin to get independent of the mother, *i.e.* when they first begin to eat for themselves. As mentioned above, this is indeed found except in the very small litters and the very large ones. In the latter case the young are longer in beginning to eat for themselves, and also owing to their stunted nature are incapable of thriving as well as other young even when they do begin to eat. Small litters, on the

other hand, grow so fast during the first fortnight on their practically unlimited supply of milk, that apparently they have no capacity for a final spurt when they begin to eat for themselves. The litter of 1, exhibiting such phenomenal growth during the first fortnight would attain unheard-of size by weaning time if the usual acceleration during the last week were shown. It would appear, therefore, that all the variations in growth may be explained satisfactorily on a basis of differential nutrition.

IV. Summary.

(1) Four hundred and seven young albino mice produced in 66 litters had an average weight of 1·41 gm. at birth and of 7·14 gm. at weaning time (3 weeks old).

(2) These average values vary very considerably, but uniformly according to size of litter. Individuals from litters of 2 and litters of 10 averaged respectively 1·20 gm. and 1·6 gm. at birth, and 4·45 gm. and 11·75 gm. at weaning time. Individuals from the intermediate sizes of litter grade fairly evenly in between these extremes. The whole growth during suckling is in fact inversely proportional to the size of litter.

(3) Growth during suckling is characterised by the active phases in the first and third weeks respectively and a comparatively stagnant period in between.

(4) These results can all be explained on a basis of differential nutrition.

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THE LEAD MINE AS AN ACTIVE AGENT IN RIVER POLLUTION

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Previous studies by the present writer(1, 2, 3) have dealt with the condition of Cardiganshire trout and salmon rivers in process of recovery from pollution by lead mines now closed: the polluted reaches may remain totally barren for some time after the mines have ceased working, but sooner or later recovery sets in, and its phases ensue as follows:

(i) Establishment of a limited Cryptogamic flora (including *Limania*, *Batrachospermum* and a few Bryophytes) and of a very sparse Invertebrate fauna consisting almost entirely of insect larvae: Oligochaeta, Mollusca, Platyhelmia and (among insects) Trichoptera still unrepresented.

(ii) Establishment of Chlorophyceous Algae and invasion by Oligochaetes, Turbellarians and caddis-larvae, as well as additional types of insect groups already represented, with fishes as occasional visitors from neighbouring waters.

(iii) Growth of flowering plants such as *Callitriche verna* and *Ranunculus aquatilis*, coupled with a large increase in Invertebrate types, Mollusca being added, and the final establishment of Fishes.

These phases may be traced successively in a single reach of the river as time elapses since closure of the mines(2), and they may also be followed through successive reaches of the same stream, passing away from the source of pollution(1). Phase (i) is associated with a constant chemical condition of the river-water—that of holding in solution small but appreciable quantities of lead (up to 0·4 mg. per litre), phase (ii) with an intermittent chemical pollution, the supply of soluble lead being renewed at flood-times, and phase (iii) supervenes only when this intermittent renewal has ceased, in some cases only after the lapse of several years. The presence of metallic solutions is believed to be the factor conditioning the barren quality of the streams: in the case of Fishes, it has been proved by experiment, first, that the flood-waters of phase (ii) are fatal in virtue of their dissolved content, second, that

artificial solutions of lead in similar concentrations are similarly fatal to these animals(3). The lethal process is probably accelerated in the streams themselves by the presence of small amounts of zinc accompanying the lead in solution. The view has been taken that these metallic solutions are formed by the action of water on small particles of galena and blende remaining in the heaps of mine-waste which are dumped by the river-side: this would account for the renewal of pollution in phase (ii) when the flooded river rises to wash the edges of the dumps.

On these observations and inferences have been based a criticism of the usual "precautionary" methods of sedimentation and screening of dumps hitherto urged upon the mine-workers, and a plea for chemical treatment of the mine-effluents to eliminate metallic solutions, but, although responsibility had been definitely fixed upon the presence of these solutions as a deterrent to the recovery of the barren streams, observations on the conditions prevalent during actual mining activity were necessary before making a final pronouncement on the prospects of finding an effective safeguard against pollution. It was conceivable that the poisonous solutions might be formed, not so much during the active phase, as after some time had elapsed for the weathering of the metallic residue in the dumps; also there might be, during activity of the mines, a fresh and totally different factor to take into account, such as the discharge into the river of finely divided earthy waste, not poisonous in character, but mechanically injurious to the life of the stream.

Since the summer of 1924, there has been an opportunity to observe the conditions set up by new activity on the part of a lead mine affecting a stream in the Cardiganshire area which is similar in natural type to those whose recovery stages have already been traced. A large lead-mining and dressing station at Esgair Mwyn, above the Marchnant Brook (a tributary of the Teifi) had been closed since 1918: the brook is said to have suffered from pollution prior to this date, but in 1923 and the spring of 1924 it exhibited the characteristic fauna of a Cardiganshire moorland stream (including trout, minnows, etc.), and chemical tests had shown that its waters contained no trace of metallic solutions, even in flood time: it therefore ranked in 1923–24 as an unpolluted stream. Activities commenced at Esgair Mwyn in the summer of 1924: by the following May the mine was working continuously, though not yet with great output, and discharging its waters along a leat into the Marchnant. In accordance with the old views on protection of rivers by "sedimentation" of mine-waste, the effluent, which emerges, turbid with waste, from the dressing-sheds, is led along a horizontal level

through a maze-like series of settling-pits dug out of the hill-side and banked with mine-sediment and earth, before it joins the clear stream from the power-house, and in these pits a very large proportion of the solid waste actually is deposited. Further deposition takes place in the leat which takes the combined flow, and which contours the hill-side for nearly a mile before reaching the Marchnant, but it is impossible to induce absolutely complete deposition, as much of the material is in the colloidal state, and in the summer of 1925 the water of the brook itself was cloudy for some little distance. Still, something like a maximum quantity of the solid material is deposited, and the old system of sedimentation may be considered to be fairly on its trial here.

Observations on the conditions which followed the opening of the mine have been made as follows:

(*a*) *Biological observations in the field.*

In the summer of 1925 a faunistic survey of the brook was made for comparison with previous surveys made in 1923 and spring 1924. Whereas the earlier surveys had shown the brook to possess the normal fauna of its type along all its length, at this time certain species were found to have disappeared altogether from the reach immediately below the leat-union. The most interesting of these species was the Gasteropod *Ancylus fluviatilis* (the freshwater limpet), which occurred on the stones not many yards above this spot, but was not found again below it. Trichopteran species disappeared in a similar way, though the occurrence of larvae of stone-flies and Ephemeridae seemed unaffected. Topographical difficulties prevented the observer from following the course of the brook for more than half a mile below this point, but it was picked up again about $1\frac{1}{2}$ miles lower, at which point the full fauna, including also trout and minnows, had been restored. (The disappearance of *Ancylus fluviatilis*, the only Molluscan species encountered with any frequency in unpolluted brooks of this acid moorland type, is peculiarly significant. In the experience of the writer, the disappearance of Mollusca is the first index to the pollution of a stream by lead-mining, while that of Trichopteran larvae marks the second phase. Far less importance is attached to the fact of seeing or not seeing fishes themselves, which, owing to their powers of swift locomotion, may be casual visitors to a reach which is unable to afford them permanent shelter. These conclusions, independently arrived at through study of Cardiganshire streams, are in entire agreement with those derived by Prof. Ortmann from his study of river pollution in Pennsylvania (4).)

(*b*) *Chemical evidence derived from analysis of samples.*

A water sample taken from the Marchnant Brook a few yards below its reunion with the leat in December 1924, though slightly turbid, was found, on analysis, to contain no trace of metallic solutions. A sample taken from the same spot in June 1925 contained dissolved metallic content as follows:

Lead	...	...	1 mg. per litre
Zinc	...	...	Nil.
Iron	...	...	A trace

Such a proportion of lead has been already proved by the writer to be fatal to fishes(3).

(*c*) *Experimental evidence derived from laboratory tests.*

1. A portion of the sample mentioned above was found by direct experimentation to be fatal to minnows, in the usual way(3).

2. A sample of the slimy deposit from the settling-pits was brought down to the laboratory. A portion of this was submitted to the County Analyst, who reported that it contained metal in the following proportions:

Lead	...	...	...	6·03 %
Zinc	...	...	...	0·33
Copper	...	...	...	0·10

Some of this material was placed in a glass tank to cover the bottom to a depth of $2\frac{1}{2}$ in., distilled water was added to a total depth of $7\frac{1}{2}$ in., and the whole was thoroughly stirred into a turbid mixture, which was kept agitated for 6 hours, then allowed to settle overnight. Liquid from the upper stratum, which was then fairly clear and only slightly opalescent, was drawn off by means of a siphon, and minnows were placed in it—the animals died in 24–36 hours, with the usual symptoms of "lead-film." A sample of this same liquid was found to have metallic content as follows:

Lead	...	...	0·75 mg. per litre
Zinc	...	...	A trace
Iron	...	...	A trace

The remainder of the liquid in the tank was then drawn off and replaced by fresh distilled water, without stirring, and two minnows were placed in the tank. By their movements, they rendered the liquid turbid, nevertheless they lived in it four to five days. During this time they were miserable objects, being thoroughly plastered with

slime, but they showed no signs of lessened activity, until the fifth day, when they were transferred to clean water and resumed normal appearance and behaviour. The water in the tank was allowed to settle for six weeks and at the end of this time a sample was siphoned off and a portion reserved for chemical analysis. One minnow was placed in the remainder of this (almost clear) sample, and two in the tank itself—all three were dead within 26 hours. Death was thus proved to be occasioned, not by the turbidity of the water in the tank, but by substances which entered into clear solution after a period of contact between the water and the slime. The sample analysed in the second instance (after six weeks' "resting" contact) contained

Lead	...	...	1·5 mg. per litre
Zinc	...	...	A trace
Iron	...	...	A trace

Repeated experiments of the same type proved that it was possible to extract poisonous metallic solutions again and again from the same sample of slime by mere contact with distilled water, and that the process of extraction was accelerated by stirring the mixture. The origin of the poisonous metallic solutions which are so important both during and after the activity of the mines is thus clearly explained: they are formed by direct action of water on particles of ore fine enough to escape with the earthy waste from the dressing-sheds, and even if all this material could be deposited safely, the water which travels over it would still carry its poisonous matter into the stream.

To summarise: the deterioration of the Marchnant after twelve months of only partial activity of the Esgair Mwyn mine, though slight and not geographically far-reaching, was none the less definite in type; doubtless it will in time become more serious, but even at an early stage it has been sufficient to substantiate certain facts.

1. Deterioration of the neighbouring stream follows inevitably upon the opening of a lead mine of the usual type, in spite of careful "sedimentation."

2. The phases of deterioration agree with the recovery phases already traced, in reverse order: the pollution while the mine is active is of the same type as the effects which persist after closure.

3. The responsible agent is the presence of metallic solutions derived by direct action of water upon the solid mine-waste: the solid waste in itself appears to be harmless, to fishes, at any rate.

The problem for those interested in the preservation of fisheries

thus resolves itself into a question of getting rid of the poisonous solutions: this question is not one for the biologist as such, nevertheless the writer's experience and reflection suggest that a solution may profitably be sought along one or more of the following lines:

(*a*) The water passing through the mine may be so treated as to reduce its plumbo-solvent power. The work of Dr J. C. Thresh on the action of water on lead(5) has shown that plumbo-solvency is largely conditioned by the presence of silicates: although this work deals only with solution from metallic lead, there is some reason to suppose that similar factors are operative in the action on galena. It is possible that a soft moorland water, such as forms the supply for many lead mines, those of Mid-Wales in particular, may be rendered incapable of action on lead ore by treating it with silica before it enters the works. Should such a treatment prove practicable, there still remains a source of danger in the trickling of water over the dump-heap, and "sedimentation" must in any case be practised with the greatest care and thoroughness in order to keep particles of ore out of the river, where, again, solution may take place.

(*b*) Solution being permitted, it may be possible to collect the whole of the contaminated waters and treat them for elimination of the metallic content before they pass into the river. The obvious suggestion is to precipitate the lead as sulphide, but the use of H_2S in this connection is not biologically defensible: it is, however, probable that a more successful method could be devised. The phenomenon of "adsorption" of metals in solution (especially lead) during filtration through materials of various kinds(6) may be helpful here, the problem being to find a filtering medium which will be effective and at the same time inexpensive for use on a large scale. This should not present insuperable difficulties; possibly even a soil bed might be efficacious to some extent.

The pressing need is to find a standard method of treatment whose use may be enjoined upon all those responsible for lead-mining operations: until this is done, the situation with regard to the law in England appears somewhat farcical. The Salmon and Freshwater Fisheries Bill of 1922 decrees that "no person shall cause or knowingly permit to flow into any water containing fish, or into any tributaries thereof, any liquid or solid matter to such an extent as to cause the waters to be poisonous or injurious to fish or the spawning grounds, spawn or food of fish": thus, owners of active lead mines are continually liable to prosecution under the Rivers Pollution Acts, but since we can suggest to them no means of rendering their effluents harmless, prosecution must either become an

empty threat or lead to the final closing of the mines. The intelligent citizen must desire to see neither river fisheries nor mining industries sacrificed, yet these are the terms of the dilemma—pending further investigations along the lines indicated.

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THE BIOLOGICAL CONTROL OF INSECT PESTS AND INJURIOUS PLANTS IN THE HAWAIIAN ISLANDS

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(With Plate XII.)

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1. Introduction.

The sugar planters of the Hawaiian Islands have set an example to the rest of the world in the reliance which they have placed on the application of scientific principles to the cultivation and protection of their crops. Their enterprise has led to the production of a yield of sugar in lbs. per acre far in excess of that raised in any other region of the globe. This achievement has had its attendant difficulties for there have been struggles of dramatic intensity between the planters on the one hand and the forces of nature on the other. Among the severest factors they have had to contend with have been the insect enemies of cane. The methods by which the latter have been and are still being subjugated form the subject of the present essay. It was the desire to obtain an insight into the fundamental principles governing this work that prompted the present writer to visit the Islands in 1925. A great measure

of profit and pleasure derived from this experience is due to the generous hospitality and assistance afforded by Mr F. Muir. Mr J. W. Waldron, of the Sugar Planters' Experiment Station Committee, arranged exceptional facilities for visiting the islands of Hawaii and Maui, and Mr O. H. Swezey rendered valuable aid in obtaining material illustrating some of the chief features of the Hawaiian insect fauna.

The Hawaiian Islands are now so well known that any detailed description is out of place here. It may be mentioned, however, that they lie 2100 miles south-west of San Francisco and at about the same distance north of Samoa. They are very isolated and are surrounded by ocean attaining a depth of 16,000 ft. Of the eight inhabited islands the largest is Hawaii which has an area about twice that of the county of Norfolk. All the islands are of volcanic origin and included in the archipelago are the highest mountains in the Pacific, Mauna Kea attaining an elevation of 13,823 ft. Of the many volcanic craters two are active, viz. Mauna Loa and Kilauea—the latter being the largest active crater in the world. The climate of the islands is cool and healthy, considering the latitude, with a mean annual day temperature of 80° F. and 68° F. at night. The rainfall varies enormously in different localities: in one region only about 12 in. fall in the course of a year while in other parts it may exceed 300 in. The staple industries of the Islands are the growing and export of sugar and pine-apples. The flora is extremely rich and is remarkable for the very high percentage of endemic genera and species. A further noteworthy feature is the extraordinary development of usually herbaceous genera into trees or shrubs. There are no indigenous land mammals while the insect fauna, like the flora, includes a high proportion of endemic forms. It is, however, perhaps even more notable on account of the absence of whole orders and major groups which are represented in most parts of the globe. Thus, there are no endemic Trichoptera, Plecoptera, Ephemeroptera or Mecoptera: there are no Chrysomelidae, Scarabaeidae or Tenthredinoidea: there are only two truly endemic species of butterfly, a single endemic species of ant and no endemic mosquitoes.

The changes that have taken place in the fauna of the Islands, through the demolition of native forests and the general spread of cultivation, have inevitably led to the extermination of many endemic species of insects. At the same time, the maritime importance of Honolulu as "the cross roads" of the Pacific has facilitated the entry, through commerce, of many alien species. A number of these immigrants are now firmly established and among them are major pests whose presence

has threatened the whole prosperity of the Islands. In view of this process of subtraction and addition that is still going on to-day, the wisdom and foresight of those Englishmen who inaugurated the *Fauna Hawiiensis*, and saw it through to completion, can scarcely be over-emphasised. This work is the scientific basis for all subsequent progress in Hawaiian entomology besides being an important contribution to our knowledge of island life in general. The part enacted by Dr R. C. L. Perkins in bringing so much of the material together and in his own personal contributions to the *Fauna* volumes are enduring monuments to his credit. The *Fauna Hawiiensis* describes and records what species are endemic to the Islands and what species are introduced, thus providing the economic worker with indispensable information. Except for the existence of this work, he would be continually at a loss to know whether he was dealing with a native or an introduced species and would be encompassed by difficulties regarding what course of action to take.

2. General Remarks on Biological Control.

Biological control of injurious insects has achieved a degree of success in the Hawaiian Islands not attained elsewhere. On the other hand, artificial methods of repression have generally proved expensive and given but poor results in that same region. Hawaiian pests are almost entirely immigrant species which have become established in the Islands, unaccompanied by the naturally occurring enemies which normally prey upon them in the lands from whence such pests originally came. The climate of the Islands is remarkably uniform, the temperature rarely falling below 60° F. or exceeding 92° F. Under such conditions hibernation is almost a non-existent phenomenon. An environment so favourable conduces to the rapid multiplication of insect pests, but it is none the less advantageous for the introduction and spread of beneficial forms. The latter can be colonised at almost any time of the year and are able to pass through uninterrupted cycles of development, owing to the abundance of their hosts and the absence of a dormant season. Furthermore, the unique character and the simplicity of the indigenous fauna, with its paucity of hyperparasites and other likely competing forms, provides a biological association offering but few obstacles in the struggle for existence.

It should not be assumed, however, that success in biological control, even in so favourable an environment as the Hawaiian Islands, has come easily, or that every beneficial insect introduced has become established in its new surroundings. The history of insect control in

these Islands, if fully written up, would have to include a number of failures and recount the expenditure of strenuous efforts and of large sums of money. Thus, the complete control of the sugar cane leafhopper only resulted after the introduction of about a score of different enemy species and after about twenty years of work. The search for effective wireworm enemies has now been maintained for several years but so far without the desired success. On the other hand, the control of the *Anomala* beetle and recent attempts to control the Avocardo mealy bug have achieved success in a remarkably short interval of time.

The earliest experiment in the introduction of a beneficial insect into the Hawaiian Islands was apparently in 1890. In that year A. Koebele forwarded a consignment of the ladybird *Novius cardinalis* from California to Honolulu for the purpose of attempting the control of the cottony cushion scale *Icerya purchasi*. The latter insect was, at that time, as destructive to citrus fruits in the Islands as it was in California. The experiment resulted in its complete subjugation and ultimate decline into insignificance. During subsequent years more than 90 different species of beneficial insects have been successfully introduced from various countries into the Hawaiian Islands and a complete list of these has recently been published by O. H. Swezey (1925). A far greater number of species, however, have failed to become established: a complete list of these is not available but a considerable proportion are on record (*vide* Swezey). In judging the success or failure of these experiments it should be pointed out that the objective is to bring about a reduction in the numbers of the pest concerned. If, for example, introductions are attempted with ten different species of parasites and only two species succeed in establishing themselves and these two are effective in restraining the host, the experiment is to be regarded as successful. It is wide of the mark to say that there were eight "failures": the object was to secure a desired degree of control rather than to ensure the establishment of all the parasites handled. A large proportion of the parasites that "failed," as Mr Muir has pointed out to the writer, could have been established if the experiments had been continued. When success has been attained in reducing a pest, such as the *Anomala* beetle, for example, by the aid of a single species of parasite or predator, further efforts to establish other species may be deemed unnecessary. Beneficial insects that have established themselves the most readily are generally those best adapted to the environment and such species usually prove the most satisfactory from the economic standpoint. With some species successful establishment has only been secured after a long

period, but generally such insects are not very effective under Hawaiian conditions.

The first entomologist to be employed on work of biological control was A. Koebele who was appointed in 1892 and served under the Territorial Government. Prior to 1904 the Sugar Planters' Experiment Station was but a small concern occupied with the study of soils and fertilisers. In that year the Station was enlarged and its scope widened. The first entomologists to be appointed on the staff were Messrs Perkins, Kirkaldy, Terry and Swezey: about that time Koebele was seconded in a consulting capacity and Muir joined the organisation in 1905. The Experiment Station began the great part which it has played in the introduction of beneficial insects from 1904. The Territorial Board of Agriculture and Forestry has participated in this work, as have also the United States Experiment Station and the Honolulu Laboratory of the U.S. Bureau of Entomology.

3. The Sugar Cane Leafhopper.

One of the most interesting and striking examples of the biological control of an injurious insect in the Hawaiian Islands is afforded by the sugar cane leafhopper, *Perkinsiella saccharicida*. It appears to have entered the Islands about the year 1897 and had increased to an enormous extent by 1902. Sugar production, for example, in a single infested plantation fell from 19,000 to 7000 tons in three years and the conversion of some of the plantations into cattle ranches was mooted. A discussion naturally arose with regard to what measures were to be adopted in combating the menace of the leafhopper and it soon became evident that, owing to local conditions and the nature of the sugar cane crop, no artificial methods were likely to meet the situation. The previous success occasioned by the control of *Icerya purchasi* and other pests, by the introduction of their natural enemies, had much influence in the adoption of biological measures. It was in 1903 that A. Koebele, acting on suggestions made by Dr L. O. Howard, made extensive observations on Dryinid and other leafhopper parasites which had been previously studied by O. H. Swezey in Ohio. This resulted in Koebele sending over a good deal of living material to the Hawaiian Islands, but technical knowledge of such parasites was at that time very scanty and the experiment resulted in much wasted effort. Most of these parasites were subsequently proved to be very restricted in their range of hosts with little likelihood of attacking the sugar cane leafhopper. In 1904 Perkins and Koebele visited Australia with the object of studying the leafhopper

in its original environment and with special reference to securing any effective parasites that were available. Perkins returned to Honolulu early in 1905, while Koebele remained in Queensland to continue the field observations. He returned from Australia later having, in the interval, also visited Fiji. The outcome of their efforts was the introduction of several parasites and predators, but certain of these failed to become established. The most promising were Chalcid parasites attacking the eggs of the leafhopper. Thus, *Paranagrus optabilis* Perks. was introduced by Perkins and Koebele from Queensland, while Koebele secured *Ootetrastichus beatus* Perks.[1] from Fiji. About eighteen months after these introductions had been accomplished, the damage entailed by leafhoppers fell to about half its original proportions and, during the following year, some 75 per cent. of the infested areas were under the influence of the beneficial insects. Outbreaks still occurred in certain districts and a condition of more complete control was aimed at. F. Muir embarked on work of a similar nature and, during 1906–7, secured the Dryinids *Haplogonatopus vitiensis* from Fiji and *Pseudogonatopus hospes* from China. It should be mentioned that other Dryinids had been previously introduced into the islands by Perkins and Koebele, but they failed to become established. The hopes that were at one time entertained of the value of this group of parasites never matured. *Pseudogonatopus hospes*, for example, was not recovered until nine years after its first introduction, while other of the species have entirely disappeared. The failure of these enemies to fulfil expectations appears to be due partly to local conditions, but more especially to hyperparasites. Some of the latter were probably native species which, owing to their minute size, had previously remained undetected, while others gained an entry into the Islands as chance immigrants brought in through commerce. It now became evident that, in order to effect the desired degree of control, some robuster type of leafhopper enemy was needed which would also withstand the bad conditions occasioned by heavy rains. An apparently promising species was secured by Muir in Fiji and Queensland. This insect was a small Capsid bug, *Cyrtorhinus mundulus* (Bred.) which lives by sucking the eggs of the leafhopper. The selection of this insect was an entirely new venture in biological control since none of the predaceous Hemiptera had been previously employed. Since the members of the family to which it belongs are almost exclusively plant-feeders this zoophagous habit was indeed exceptional. Muir's observa-

[1] *Ootetrastichus formosanus* Timb., introduced by Muir from Formosa, in 1916, has also contributed to the decline of the leafhopper.

tions, however, showed that the habit was apparently a fixed one, nevertheless the lurking possibility of the insect varying its behaviour under new surroundings had to be watched for. The introduction of the *Cyrtorhinus* was effected in 1920 and, at first, it seemed doubtful whether it was going to maintain itself. Its subsequent sudden increase and spread allayed these fears and it is now generally conceded that the *Cyrtorhinus* is the one species which has completed the subjugation of the leafhopper to-day. The effects of the activities of this bug are truly remarkable and during the writers' visit to the Islands it proved difficult to find many examples of the cane pest which was once so abundant and so destructive.

It is evident that, with the introductions of the new components thus recounted, the biological association that has become centred around the leafhopper has greatly increased in complexity. It is always possible that unforeseen factors may in the future upset the balance thus established, and bring about conditions favourable to the multiplication of the leafhopper, thus occasioning local outbreaks of that pest. It is occurrences of this nature that need constant watching for. A prevalence of strong winds in one direction might easily result in the accumulation of a small number of surviving leafhoppers in sheltered cane fields. If followed by torrential rains such delicate egg-parasites as *Paranagrus* would probably suffer severely and a local reinfestation by the leafhopper result. A heavy tax might then be placed upon the capacity of the *Cyrtorhinus* to cope with the situation, and a condition demanding fresh efforts arise.

4. The Sugar Cane Borer.

The sugar cane borer, *Rhabocnemis obscura* (Boisd.), was the next pest which received attention and it had been known to damage cane in the Islands as early as 1865. The initiation of a project for the control of this beetle was in 1906 when F. Muir journeyed to the Orient, mainly for purposes of investigating other sugar-cane insects. While in that part of the world he also made a search for the cane borer in Southern China, the Malay States and Java but without success, although some material of possibly useful predators was forwarded to Honolulu. Muir then decided to proceed to West Borneo where there appeared to be good chances of meeting with the insect. Unfortunately the result was negative and from there he proceeded to Amboina and afterwards on to Larat by way of Kei and the Aru Islands. The beetle was ultimately found in great numbers in Larat breeding in pinang and sago palms.

With the experience and fresh information thus obtained Muir returned to Amboina and discovered that the insect existed there in sago palms and was attacked by a Tachinid parasite *Ceromasia sphenophori* Vil. The first stage in the work was thus attained and there then began the experiment of transporting the parasite from Amboina to Honolulu. Owing to paucity of communications it was necessary to transmit the material *via* Hong Kong and Japan. F. W. Terry was, therefore, sent from Honolulu to Hong Kong to receive the material from Amboina and to attempt the breeding of the fly there. Owing to delays on the voyage, the insects perished before reaching Hong Kong and it eventually became necessary to discover some other locality where the parasites existed from whence it would be easier to forward them to Honolulu. After visiting Ceram, Muir proceeded to New Guinea where both the beetle and its parasite were discovered. With a number of cages stocked with borer larvae, in which the parasite had deposited its eggs, Muir set out for Honolulu *via* Brisbane. On arriving at the latter port he unfortunately had to go into hospital with typhoid. This entailed the sending forward of the cages to Honolulu unattended and all the parasites succumbed before reaching their destination. On recovering from sickness, Muir returned to Honolulu in order to spend some time recuperating in the mountains and, in January, 1910, he again set out for New Guinea *via* Brisbane. Owing to the shortness of the life-cycle of the Tachinid, and difficulties attending the breeding of the insect in small cages, it was decided to establish intermediate breeding stations in Australia and in Fiji. At Brisbane Muir arranged to meet J. C. Kershaw and provide for the erection of suitable breeding cages at Mossman. This work being accomplished, he journeyed to New Guinea and made ready a large consignment of parasitised borer larvae, together with puparia of the Tachinid, to be taken personally to Mossman. There were serious delays owing to weather, and other causes, before the destination was reached and much valuable material was lost as the result. Sufficient parasites were bred out, however, at Mossman for Muir to take to Fiji where he had previously arranged for the erection of a suitable cage for their reception. Muir had left New Guinea with an attack of malaria which continued while he was at Mossman and he ultimately had to go into hospital in Fiji, but not before he had transferred the Tachinids into their breeding quarters. Kershaw arrived in Fiji later, bringing with him a further stock of parasites bred in Mossman, and the final result was that Muir, and subsequently Kershaw, succeeded in conveying the parasites bred in Fiji to Honolulu. It was in the summer of 1910 that

the second stage of the work was accomplished, but it was only after surmounting great difficulties. It now remained to establish the Tachinid in the Hawaiian Islands and ensure its distribution in all the borer-infested plantations.

A certain number of the flies were directly liberated in the cane fields, as they appeared in the breeding cages, while others were retained for building up an increased stock of the insect. In order to breed the parasites successfully it was necessary to have large cages in the open air. The host larvae were inserted in artificially-made holes in sugar-cane stems, one to each internode, the holes being stopped with frass from the larval burrows. The larvae were either gathered direct from the fields, or bred in large tubs, in which many lengths of soft cane were placed along with the beetles. When the appropriate conditions were attained, from 50–75 Tachinids were introduced into a cage. These freely parasitised the borer larvae and, when the Tachinids attained the pupal condition, their puparia were placed in suitable types of receptacles out in the plantations where the flies were due to emerge. It is remarkable that such small numbers as 100–250 puparia often proved sufficient to colonise average-sized plantations. In one instance recoveries were made nine months after liberation of the Tachinids. The latter were readily found and had spread from $\frac{1}{2}$ to $1\frac{1}{2}$ miles in the adjoining fields, having passed in the meantime through five or six generations. By the end of 1913 the beneficial insect had become firmly established in 39 different plantations and, in that year, there was a noticeable reduction not only of the borers in the fields, but also in loss of the crop harvested. In one plantation the number (in ounces) of beetles collected fell in two years from 27,010 to 1568. There appears to be no doubt that, at the present day, the Tachinids have attained their maximum efficiency. Owing to the great reduction in the number of borers present there is a corresponding scarcity of parasites. In many localities indications of borer injury are hard to discover and the parasites correspondingly difficult to find, but they are nevertheless present. At times local outbreaks occur and, in certain districts, heavy rainfall and high winds, coupled with a variety of cane that lodges, the effectiveness of the Tachinid is much less than elsewhere and the borer gains the ascendancy. Efforts to secure other parasites from Mexico in 1923 were not successful and attention is now being given to the East Indies in the search for further beneficial insects.

5. The *Anomala* Beetle.

In the case of the Lamellicorn beetle, *Anomala orientalis*, a remarkably complete degree of control has been achieved. This insect was probably introduced in potted plants when it entered the Territory prior to 1910. Fortunately, the infestation is a localised one as the larvae, when abundant, nearly or quite kill out the cane. It has been observed that the female beetles commonly deposit their eggs prior to flight which fact seems to explain its restricted distribution. Attempts have been made to restrain this insect by importing about twelve species of parasites from several countries, but the only kind that has become established is the solitary wasp, *Scolia maniliae*. This insect was obtained by Muir in the Philippines and he transmitted the first consignment to Honolulu in November, 1915. The early shipments were small and gave poor results, but with the assistance rendered by H. T. Osborn, and later by F. X. Williams, it became possible to send over material on a more considerable scale. In September, 1916, the first recovery of the *Scolia* was made in the field and, by June, 1917, it had established itself over a considerable part of the area infested by the *Anomala*. It occupies to-day a zone far beyond that appropriated by its host, it being found in varying abundance on most of the larger islands and in localities where the *Anomala* is wanting. In these situations it maintains itself by parasitising larvae of the allied beetle *Adoretus sinicus*, which entered the Islands a number of years previously from Japan. There is no doubt that *Scolia maniliae* has proved to be a most beneficial species and its activities are such that the larvae of the *Anomala* are sufficiently restrained from increasing to their former destructive proportions. The beetle has been confined to the region which it has occupied for several years past and although, at the present time it is very slowly spreading, it is doing so in small numbers only.

6. The Avocardo Mealy Bug.

The Avocardo mealy bug, *Pseudococcus nipae*, has for a long time past been a serious pest. It infests bananas, alligator pears, figs, guavas, etc., causing the leaves to fall several times in the year. Its control has been achieved only quite recently through the efforts of H. T. Osborn in Mexico. This entomologist was able, in 1922, to secure material of an internal parasite, *Pseudaphycus utilis*, belonging to the Chalcid family Encyrtidae. This insect, in the relatively short period of about two years, has proved remarkably effective and in many localities at

the present day the mealy bug is difficult to find. Two Coccinellid beetles, *Hyperaspis silvestrii* and *Curinus coeruleus*, were also brought over at the same time and have played some small part in the control of the same insect. These beneficial insects are now well established on the islands of Oahu, Maui and Hawaii.

7. Pyralid Leaf-Rollers.

The indigenous Pyralid leaf rollers *Omiodes accepta* (Butl.) of the sugar cane and *O. blackburni* (Butl.) of palms are among the very few native insects of injurious propensities. They have, in the past, caused considerable damage but at the present day they are no longer regarded as pests of any serious consequence. They are parasitised by a considerable number of enemies, some of the latter being indigenous, but the most efficient pertain to species that have been introduced. In some seasons and in certain localities, outbreaks of *Omiodes* occur and it appears that, on such occasions, the prolific breeding of these moths temporarily enables them to outstrip the restraint exercised by their parasites. It is noteworthy that none of these parasites, whether indigenous or introduced, confine their activities solely to the *Omiodes*, but affect other lepidopterous hosts also. The most important parasite is considered to be the Braconid *Macrodyctium omiodivorum* Terry, which was probably introduced by Koebele from Japan in 1895. As many as 75 per cent. of the larvae of *O. accepta* have been known to be destroyed by this species. *Chalcis obscurata* Walk. was likewise brought in by Koebele from Japan at the same time and from China a year later. It very quickly became established and is now common throughout most of the islands. The well-known Chalcid *Trichogramma pretiosa* Riley was introduced by Koebele from the United States prior to 1900. No detailed studies have been made as to the intensity of its parasitism but it is the only egg-parasite affecting the *Omiodes*. The Tachinid *Frontina archippivora* (Will.) probably got accidentally imported from the United States and has subsequently adopted a wide range of lepidopterous hosts in the Hawaiian Islands. It can only be regarded of accessory value in so far as *Omiodes* is concerned and it has not been possible to gather any information regarding the extent to which it parasitises that genus.

8. The Mediterranean Fruit Fly.

The foregoing remarks only refer to pests which have been, or are being, largely controlled by means of imported natural enemies. There

are, on the other hand, certain other pests over whose activities economic control has not yet been achieved to a satisfactory degree. The most important of these pests is the Mediterranean fruit fly, *Ceratitis capitata*. This insect was discovered in the islands in 1910 and had probably made its entry a year or two previously from Australia. Various attempts at control were made, including insecticidal treatment, but without success. The great abundance and variety of the host fruits, over 70 kinds being found in the vicinity of Honolulu alone, and the fact that some flourish at all seasons of the year was the main cause of the failure of the efforts made. In 1912 Prof. F. Silvestri was engaged by the Hawaiian Board of Commissioners of Agriculture and Forestry to search for fruit fly parasites. Silvestri mainly concentrated his observations in Africa but also visited Australia. He arrived at Honolulu in May, 1913, with five species of African parasites and one species from Australia. This work resulted in the effective colonisation of two species, viz. the Braconids *Opius humilis* Silv. from S. Africa and *Diachasma tryoni* Cam. from Australia. In 1914 the Territorial Government commissioned D. T. Fullaway and J. C. Bridwell to visit West Africa in order to endeavour to obtain other parasites. The result of their efforts was that the Braconid *Diachasma fullawayi* Silv. and the Chalcid *Tetrastichus giffardianus* Silv. were successfully established. All the four above mentioned species are now widely distributed through the Islands but the net result of their activities is insufficient to give the required degree of control. It is noteworthy, however, that before the parasites commenced their activities it was almost impossible to obtain guavas free from larvae of the fruit fly, but now it is only a small minority that are infested. The same results have not been achieved with regard to many other fruits and this circumstance appears mainly to be due to two factors. Firstly, the *Ceratitis* larvae occur in many fruits which have a thick pulp which prevents their being reached by the parasites already destroying the larvae occurring in thin-pulped fruits. Secondly, the four introduced parasites are to a considerable extent competing against one another. Thus, when *Opius humilis* occurs in the same host larva as any one of the other three species of parasite, it is invariably killed. At first *O. humilis* was the most effective of the four enemies but, during the past nine years, its parasitism has steadily fallen from 31 per cent. in 1915 to 4·1 per cent. in 1923, while the other species have fluctuated in value very considerably. The total combined parasitism of the four species, however, has remained around the region of 43 per cent. to 51 per cent. during the four years ending 1923. It is evident, therefore,

that although the above-mentioned parasites do not at present adequately control the fruit fly in Hawaii, they have decreased the infestation to a marked degree. It remains an open question whether further adjustment will take place in the inter-relations of the parasites, to the extent of raising the sum total of the infestation, or whether efforts should be made to introduce other parasites. The view has been expressed that if the biology of these parasites had been thoroughly studied, prior to liberation, their mutual relations would have been disclosed and *O. humilis* only would have been liberated. It is claimed in some quarters that this single species of parasite would have proved a more efficient agency than the combined activities of four different species as at present.

9. The Australian Fern Weevil.

Perkins mentions that the Fern Weevil, *Syagrius fulvitarsis* Pasc., was first observed in 1900 in fernhouses in Honolulu on maiden-hair ferns only. At that time the possibility of its spreading to the tree ferns of the indigenous forests was not anticipated. In the years that followed it noticeably increased in abundance and, extending its range, has more recently attacked the native fern forests around Kilauea in Hawaii Id. The *Sadleria* fern has been threatened with total extinction and the destruction of this, along with other species, would involve serious consequences to water conservation owing to the shelter afforded by dense growths of fern. Early in 1921 C. E. Pemberton visited New South Wales, which was presumed to be the home of weevil, and the insect was sparingly found in its natural habitat. The conditions prevailing in the virgin forests of that country are not unlike those obtaining in the fern forests of the Hawaiian Islands. This fact supported the conclusion that the introduction of any available natural enemies from that region into the Territory of Hawaii offered a good prospect of success. While in Australia, Pemberton succeeded in discovering the Braconid, *Ischiogonus syagrii*, which was maintaining a parasitism approaching 60 per cent.—a feature which appeared to be responsible for the comparative scarcity of the *Syagrius* in its natural habitat. Two consignments of the parasite sent to Honolulu yielded 319 examples and these proved sufficient to start its colonisation in the Islands. At the present time the weevil is being very considerably checked and, although still spreading to a small extent, it is not occasioning damage to its former extent. It is a matter for speculation whether, in the event of thorough control of the weevil being maintained, the *Sadleria* will

be able to reassert itself. It may be mentioned that no examples of that fern were seen when the writer, in company with Mr Muir, spent a day in the Kilauea district and neighbouring region of the island.

10. Other Insect Pests.

Coccidae and Aphides. Only incidental reference has so far been made to the early work by Koebele. These efforts were largely concerned with the introduction of various Coccinellidae with a view to the control of certain scale insects and aphides. The introduction of *Novius cardinalis* has already been alluded to (p. 405). In 1894–5 Koebele went on an extensive tour in Australia, Ceylon, China and Japan in search of beneficial insects, and a great deal of material was brought to Honolulu. Although a number of the species subsequently failed to maintain themselves in the Islands, other proved successful. The success of the latter, in fact, appears to have been so complete that little is now heard of the pests in question. Of the species that were introduced, became established and remained effective, the most important are *Cryptolaemus montrouzieri* Muls. and *Coelophora inaequalis* (Fabr.). The first-mentioned species was introduced from Australia into California and reached Honolulu *via* the latter country. Its great efficiency is recorded by Perkins (1925) who remarks that "In 1892, throughout the Kona district, where I was then stationed, *Pulvinaria* covered many of the trees, which were in a dying condition and many, in fact, were dead. After the *Cryptolaemus* was taken there, this scale to a large extent disappeared. There may be some who still remember the strange appearance of the trunks of many of the larger ornamental trees in Honolulu in June, 1896, when the *Cryptolaemus* larvae, having become full-grown, congregated together so as to form large white patches covering several square feet of the surface, which was entirely hidden by them. Owing to the white covering of the larvae many people mistook these for scale insects and were actually destroying them."

The *Coelophora* was introduced from Ceylon, Australia and China. That it proved not less effective, as regards the destruction of aphides, may be gathered from the following remarks by that same authority. "In June 1895, many of the native trees in the kipukas that are found in the forest at various parts within eight or ten miles of the crater of Kilauea were covered with a conspicuous black Aphid and others less noticeable. Many of the *Pelea* trees, especially, were in a dead or dying condition, but other forest trees were affected. At this time single specimens of *Coelophora* were rarely noticed, having evidently just

arrived at the locality. In September, the ladybirds were in thousands and when the same places were revisited in August, 1896, we were unable to find even a single specimen of the large black Aphid."

Other ladybirds introduced and successfully established included *Rhizobius ventralis* Erich., introduced from Australia *via* California, preying upon mealy bugs: *Orcus chalybeus* (Boisd.), introduced from Australia, preying upon Diaspine scale insects and *Platyomus lividigaster* Muls., also introduced from Australia, preying upon aphides. In addition to the afore-mentioned species there were others less conspicuously successful, some failing to increase sufficiently as to be of noticeable value. It has been, however, the combined work of a number of species that has greatly restrained such pests as coccids and aphides. It is necessary also to mention that thirty or more species of minute Chalcid parasites have been introduced by Koebele and others to prey upon scale insects, and with respect to these Perkins remarks: "No record exists of the minute scale parasites which were frequently sent. For instance, it is quite uncertain whether some of the established parasites on scales were the results of his sendings or whether they came by other means, but it is hardly probable that of the large number of species sent none survived, even though they had no expert handling on arrival."

Cutworms and Army worms. Cutworms and army worms number thirty-five or more species in the Hawaiian Islands. The majority are indigenous to the fauna and only four native species have, so far, become pests in the cane fields. Of the introduced species, the army worm (*Cirphis unipuncta*) is undoubtedly the most serious enemy, particularly to fodder crops and to the young growing cane. The ravages of these insects are partially checked by the use of poisoned baits but their natural enemies are the most important controlling agents. The Indian Mynah is a bird that was introduced by the botanist Hillebrand about 60 years ago for the express purpose of reducing the numbers of these insects and it has done good service without becoming undesirably abundant. Severe outbreaks of both army worms and cutworms still continue and efforts have recently been made to ensure a greater degree of control. In 1923 H. T. Osborn sent over consignments of the Chalcid *Euplectrus platyhypenae* from Mexico, and by the following year nearly 46,000 examples were bred and liberated in the Islands. It is, however, too early to know the extent to which the parasite has adequately established itself but it has been found wherever it has been searched for in the Hawaiian Islands. The breeding and liberation of the insect are still being carried out.

Wireworms. Wireworms, especially *Monocrepidius exul* Shp. and *Simodactylus cimamomeus* (Boisd.) are perennial enemies of sugar cane, and, notwithstanding persistent searches made for parasites, no effective enemies have so far been secured. Mention should be made of the work of F. X. Williams in the Philippines, Panama, Ecuador, Brazil, and British Guiana; of Pemberton in Fiji and Queensland and of H. T. Osborn in Mexico. Although these efforts were unsuccessful in so far as wireworm enemies are concerned, they resulted in the collection of useful information relative to sugar cane insects and other conditions in the countries visited.

The Horn Fly. This insect is a most persistent and troublesome enemy of livestock. Attempts so far made by the introduction of other species of insects to enter into competition with it have not led to any noticeable degree of restraint. The insects introduced included not only predators and parasites but also coprophilous forms, various species being obtained, mainly through Koebele's efforts, from Mexico, Arizona, Australia and Germany. Among the few that succeeded in maintaining themselves was the Cynipid *Eucoila impatiens* Say, an Arizona parasite of Dipterous larvae in cow-dung. The problem of the control of the horn fly is still under consideration and it has been suggested that Africa is the most promising land to look to in future for parasites.

In addition to the search for further parasites of the cane borer, wireworms and horn fly, other projects are in hand either by the combined or separate efforts of the H.S.P.A. and the Board of Agriculture and Forestry. These include the control of the pine-apple mealy bug (*Pseudococcus bromeliae*), the so-called Japanese beetle (*Adoretus sinicus*) especially destructive to rose trees, and the mole cricket. Problems under discussion, and which will presumably form part of a future programme, include the control of the cane aphis, the cane mealy bug (*Pseudococcus sacchari*) and the corn aphis (*Aphis maidis*) which is concerned with the spread of mosaic disease.

Some idea of the vigilance that is needed to preclude the accidental entry of still further pests into the Islands may be gathered from recent reports of the Chief Plant Inspector, Mr E. H. Ehrhorn. During the biennial period, January 1st, 1923–December 31st, 1924, 1627 vessels entered the ports of the Islands. They carried vegetable matter, subject to inspection, numbering nearly 983,000 packages, while 5219 postal packages were also examined. The importance of this side of control work is fully recognised by the provision of a well-organised inspection department and the best equipment money can procure.

11. The Control of Injurious Plants.

The first attempt to control a noxious plant by its insect enemies was made in the Hawaiian Islands. Nearly 70 years ago Dr Hillebrand, the distinguished botanist of the Islands, introduced the thorny species of *Lantana*, *L. camara*, for ornamental purposes. This shrub subsequently spread to such an extent that it became a veritable scourge to the pastures of low-lying regions, especially on the leeward side of the Islands. It is a native of the warmer parts of America, and during a visit made by Koebele to Mexico in 1898 it was observed in its habitat and some insects were bred from *Lantana* seeds. In 1902 Koebele again visited Mexico, this time at the request of the Hawaiian Board of Agriculture and Forestry. He set out with the object of securing any promising insect enemies of the plant for purposes of introduction into the Islands. The result of his work was that 23 species of insects were sent to Perkins in Honolulu and of these eight were successfully established. Each of these eight species attacks the *Lantana* in a somewhat different way. The most effective appears to be the Tortricid moth *Crocidosema lantana* Busck., whose larvae bore into the flower stems and consume the flowers and fruit. The larvae of the seed fly *Agromyza lantanae* Frogg. attacks large numbers of the ripening berries, either destroying the seeds or causing the berries to shrivel up on the bushes. The Tingid bug *Teleonemia lantanae* Dest. destroys the young leaves so effectually that extensive areas of *Lantana* are checked in growth and fail to blossom. The larvae of the Trypaneid gall-fly *Eutreta xanthochaeta* Ald. to some extent checks the growth of new shoots. The larvae of the plume moth *Platyptilia pusillidactyla* (Walk.) destroy many of the flowers in very much the same manner as those of the Tortricid already referred to; on the whole it appears to be less efficient than the latter insect. The Tineid leaf miner *Cremastobombycia lantanella* Busck. is of minor importance but its larvae when abundant cause the premature death of the leaves and to some extent restrain the vigour of the plant. Finally two species of Lycaenid butterflies *Thecla echion* L. and *T. bazochii* God. are important since their larvae destroy a great many of the flowers and thus restrain the production of the berries. Unfortunately the Chalcid *Pantarthron flavum* Perk. parasitises the eggs of the Theclas to some extent, while on the other hand the larvae of *T. echion* have proved not entirely harmless to garden plants, being occasionally found feeding on fruits of the egg-plant. The most valuable of the insects just enumerated are those which inhibit seed-production, thus minimising the further spread

of the *Lantana* and of its reinfesting land from which it had been eradicated. By the year 1905 the introduced insects had become generally distributed through most of the islands. The increase of some of the species in their new surroundings was phenomenal. Thus Perkins mentions that only three months after the last insects were liberated the *Agromyza* was already in countless numbers and along a mile strip of abundant *Lantana* in one locality it was hardly possible to find a sound berry. The various introduced insects have proved most effective in the drier regions, and in such localities at the present day it is difficult for a visitor to form an adequate conception of the original infestation by this shrub. For many miles in different directions around Honolulu the writer found that all that is evident is the presence of scattered bushes along the roadsides and here and there a denser growth on rough lands adjoining the coast. In the island of Hawaii there are, however, large areas on the sites of some of the older lava flows. The presence of the plant under these conditions does not appear to be directly harmful since little else will grow in such situations and the roots of the *Lantana* are evidently aiding the conversion of the lava into soil. In Maui Island the plant is controlled very much in the same degree as in Oahu Island. In addition to the insects already enumerated the scale *Orthesia insignis*, although a dangerous pest of polyphagous habits which has been accidentally introduced, shows a marked predilection for *Lantana*, killing out the latter in places. It is also worthy of note that where *Lantana* grows down to the shore it becomes killed out apparently by the salt spray driven inland by the sea.

Another noxious weed which has gained a footing in the Islands is Pamakani (*Eupatorium glandulosum*) which is also native to Mexico. It first appeared on Maui Island, where it has now become a serious pest on the ranch lands, besides being established on the islands of Oahu and Molokai. In May 1923 H. T. Osborn, who was at that time already engaged in entomological work in Mexico, was requested to investigate the occurrence of the plant in that country and the possibility of controlling it in the Hawaiian Islands. His observations indicated that Pamakani is troublesome on the Mexican ranches but under greater restraint than in Maui Island. The relative values of the different factors involved in its control are possibly somewhat complex, but it appears that competition with other plants probably plays a much more important part than insects. Upwards of 50 species of the latter were found by Osborn to attack this weed and it is possible that certain of these might prove more effective under Hawaiian conditions

than in Mexico. It is not, however, advisable to attempt the introduction of any of these forms until their bionomics has been more adequately investigated.

The obnoxious tropical sedge known as nut grass (*Cyperus rotundatus* L.) probably entered the Territory about the roots of imported plants and has since become a pest plant. Efforts are being made to introduce and establish a weevil and a Tortricid from the Philippines with a view to its control. A small consignment of these insects has been received in Honolulu and the experiment will be watched with considerable interest. Exceptional care is obviously required in order to ascertain whether these imported beneficial insects are likely to remain specific to their host plant, under a new environment, and not become enemies of forage grasses, etc.

The writer observed considerable areas of furze over grazing land on the slopes of the mountain Haleakala on Maui Island. It was not possible to gather any information relative to the incidence of this plant, but it appears not unlikely that it may, in future, become a pest of importance in the absence of any efforts at control.

12. Summary.

1. Insect pests in the Hawaiian Islands are, with few exceptions, immigrant species from other countries. Their control by biological methods dates from 1890, when A. Koebele introduced *Novius cardinalis* for repressing the cottony cushion scale, *Icerya purchasi*, and the experiment was completely successful.

2. The sugar cane leafhopper, *Perkinsiella saccharicida*, has been suppressed by the combined activities of several imported species of Chalcid egg-parasites, followed by the introduction of the predaceous Capsid, *Cyrtorhinus mundulus*, from Fiji and Queensland.

3. Satisfactory control of the sugar cane borer, *Rhabocnemis obscura* has been achieved in most infested areas by the Tachinid *Ceromasia sphenophori*, introduced from New Guinea. In certain districts, under bad climatic conditions, outbreaks still occur and efforts are being made to meet the situation by introducing other beneficial insects.

4. Efficient control of the beetle, *Anomala orientalis* has been obtained by the introduction of the wasp, *Scolia maniliae* from the Philippines. This pest has not since increased to its former destructive proportions.

5. The Avocardo mealy bug, *Pseudococcus nipae*, affords one of the most recent examples of biological control. Since the importation of the

Chalcid parasite *Pseudaphycus utilis* from Mexico in 1922 this pest has been difficult to find in many districts.

6. The indigenous Pyralid leaf-rollers *Omiodes accepta* and *O. blackburni* are no longer pests of serious consequence. They are controlled by several enemies, chief among them being the Braconid, *Macrodyctium omiodivorum* and the Chalcid, *Chalcis obscurata*: both parasites were originally obtained from Japan.

7. Satisfactory repression of the Mediterranean fruit fly, *Ceratitis capitata*, has yet to be achieved. The importation of parasites from Africa and Australia has markedly decreased the infestation, but their efficiency appears to be partly neutralised by the species competing against one another and supra-parasitism is prevalent.

8. The Australian fern weevil *Syagrius fulvitarsis* no longer occasions damage to tree ferns to its former extent. The recent introduction of the Braconid *Ischiogonus syagrii* from Australia has brought about this result.

9. Early introductions of various Coccinellidae, more particularly of *Cryptolaemus montrouzieri* and *Coelophora inaequalis*, resulted in the complete subjugation of certain coccids and aphides.

10. Promising results are being obtained against army worms and cutworms by the recent introduction of the Chalcid *Euplectrus platyhypenae* from Mexico. The breeding and liberation of this parasite are still being carried out.

11. Control of wireworms and the horn fly has not, so far, met with success.

12. The colonisation of a number of species of insect enemies of *Lantana*, from Mexico, afford the first example of pest plant control by this method. The restraint which they have exercised, more especially by prevention of seeding, has checked that plant reinfesting areas previously cleared.

13. The repression of nut grass (*Cyperus rotundatus*) by the introduction of insect enemies from the Philippines is being carried out as a preliminary experiment and a survey of the possibility of controlling Pamakani (*Eupatorium glandulosum*) has been made in its Mexican habitat.

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Fig. 1.

Fig. 2.

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EXPLANATION OF PLATE XII.

Fig. 1. Harvesting sugar cane, Hawaiian Islands.

Fig. 2. Main building, Hawaiian Sugar Planters' Experiment Station, Honolulu.

(Photographs supplied by courtesy of the Hawaiian Sugar Planters' Association.)

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STUDIES ON CONTACT INSECTICIDES

PART IV. A QUANTITATIVE EXAMINATION OF THE TOXICITY OF CERTAIN PLANTS AND PLANT PRODUCTS TO *APHIS RUMICIS*, L. (THE BEAN APHIS)

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(With 6 Text-figures.)

Renewed attention has been given in the last few years to the investigation of the insecticidal properties of plant materials, in the form of extracts or powders. Roark(1) in 1919 put together a great deal of the available information on the subject; and the list of plants which have been reported to possess insecticidal properties, or which have been actually tested, has been extended and brought up to date by McIndoo and Sievers(2), who also include in their paper some new experimental data.

Perhaps the most interesting plants which have been definitely proved to have a marked toxic effect upon insects, are some which are used by the natives of various tropical countries as fish poisons. The best known of these is Derris or Tuba Root (the roots of *Derris elliptica* and *D. uliginosa*), which has been in actual use as an insecticide for some few years. Extracts of another plant used as a fish poison in Peru, known locally as "cube," are reported by McIndoo and Sievers (*loc. cit.*) to have considerable toxicity to a variety of insects, especially Aphides; unfortunately the botanical identity of the plant is uncertain. More recently, in Part II of this series of papers on Contact Insecticides, we have given an account of experiments with aqueous and alcoholic extracts of the leaves, stems and seeds of an African fish poison, *Tephrosia Vogelii* Hook. We have now been able to obtain samples of other species of *Tephrosia* and of several other tropical plants used for poisoning fish, and in the present paper we give the results of quantitative experiments on the insecticidal value of extracts of these plants, and of certain toxic substances isolated from the extracts, again using *Aphis rumicis* as our

standard test insect. We have also incorporated data obtained by the investigation of certain other plants (which, so far as we are aware, have not been used as fish poisons) and of a few alkaloids and extracts of the plants from which they have been derived.

We wish to express our most sincere thanks to Dr Martin Leake and his collaborators, Mr W. G. Freeman, Prof. H. Ballou and Mr J. Jones, for several West Indian plants used as fish poisons; to Mr R. E. Massey for a supply in bulk of *Tephrosia Vogelii*; and especially to Mr R. A. Altson of the Botanic Gardens, Georgetown, British Guiana, for large samples of Black and White Haiari, *Tephrosia toxicaria*, *Clibadium asperum*, *Sophora tomentosa* and *Asclepias curassavica*.

Methods.

The methods we have employed have been set out in detail in Parts I and II of this series of papers(3, 4) and little reference need be made to them here. Where, however some departure from the usual technique was rendered necessary it is indicated in the appropriate section. *A. rumicis* (adult apterous agamic females) was used as a test subject and the spraying tests in the laboratory were carried out in the apparatus already described by two of us(5). The results obtained are as far as possible strictly comparable, but it should be pointed out that the method is designed to procure a maximum of efficiency under such conditions that each insect receives the same full dose of spray.

The data are expressed in both diagrams and tables. As mentioned in previous papers, the curves drawn through the various points must not be regarded as being suitable for mathematical analysis; such is not the case; they are to be looked upon as tracing the course of events when, under the conditions of our experiments, a comparatively small number of insects are sprayed with a series of concentrations of the material in question. We have averaged the results when a substance has been tested at the same concentration on different occasions.

Later experience has shown us that, in certain cases, the accuracy with which it is practicable to make up liquids of very low concentration is not such as to warrant us in differentiating between concentrations lying very close together. In such cases, we have averaged the results and plotted an intermediate point on the diagram.

The scale to which the diagrams are drawn is not the same in all cases; it is varied according to the toxicity of the materials dealt with.

Plants used as Fish Poisons.

(A) *Genus* Lonchocarpus. We have received from Mr R. A. Altson samples of parts of two plants, known as Black and White Haiari, which are extensively used by the natives of British Guiana for the purpose of catching fish. They are climbing plants (lianes or "bast-ropes") and belong to the Leguminosae but there is some uncertainty as to their precise identification. Mr Altson informs us that they are probably species of *Lonchocarpus* and that Haiari was identified by Schomburgk as *Lonchocarpus densiflorus* Benth.[1]. Schomburgk apparently did not distinguish between White and Black Haiari but there is reason to believe that his identification referred to White Haiari. This cannot however be verified in the absence of complete specimens of the two plants and we are not able to say at present whether they are two distinct species of *Lonchocarpus* or varieties of the same species.

Mr Altson also gave us the following interesting description of the manner in which the plants are used by the natives. "The *roots* of White Haiari and the *stems* of Black Haiari are beaten out into a horsetail and shaken into water. This water is then thrown into the creek and in about ten minutes the fish in that area are stupefied or killed and float to the suface. The roots of Black Haiari are said by the Indians to be of little use as a fish poison."

[1] The following quotations from *Travels in British Guiana*, 1840–1844, by Richard Schomburgk, translated by W. E. Roth (Georgetown, British Guiana, 1922–1923), are of interest.

"...the very important *Lonchocarpus densiflorus* Benth., with the milky pungent root-juice of which these people [the Indians] stupefy fish. When the Indians want to carry out a poisoning expedition, they smash and squash up the roots beforehand with huge wooden clubs and throw the mass into the water at those spots where they notice the fish to be plentiful; in about 10–15 minutes its effects are visible upon the scaled denizens. They rise to the surface, spring out of the water, gasp for breath, and then turn belly upwards, in which condition they are caught by hand or shot with the arrow. The small finger-long fry usually die while the larger fish generally recover after a time. Besides the *Lonchocarpus densiflorus* the Indians also use for the same purpose the *Tephrosia toxicaria*, *T. cinerea* Pers., *Phyllanthus Conami*, *Clibadium asperum* De C.: none of these plants shows itself as effective as *Lonchocarpus*" (vol. I, p. 272).

"As the *Lonchocarpus densiflorus* Benth., grew in fair quantity close to the sources of Pirara, poisoning of the water by the milky juice of its roots was the method especially adopted for catching fish. That the poison not only acts upon the respiratory organs as can be recognised from the difficulty in gasping for air and widely opened gill-covers, but that it affects the nervous system to an equal degree is shown by the generally dilated pupils of the dying fish" (vol. I, pp. 319–320).

"...*Tephrosia toxicaria* Pers. called Yarro-conally by the Indians and used by them for poisoning the Yarro Fish (Hypostomus or Doras?) which is immune to the milky juice of the Haiary (*Lonchocarpus densiflorus*)" (vol. II, p. 120).

The stems of Black Haiari and root and stems of White Haiari were tested for their insecticidal properties[1]. Ten gm. of the finely divided root or stems were soaked over night in absolute alcohol, filtered through fine muslin, and made up to 100 c.c. with the washings with absolute alcohol. The alcoholic extract was diluted as required with 1 per cent. saponin solution and used for the spraying tests. Controls were carried out with solutions of alcohol at concentrations much higher than those used in the case of the extracts; no deleterious effects on the insects were observed.

This method is not identical with that used in our earlier work on *Tephrosia* (3) where the extracts were taken to dryness and re-dissolved before use. The importance of establishing a standard method for the preparation of spray fluids from plant extracts for quantitative tests of this nature is evident, as has been pointed out by F. Willaume (6), and an investigation on this point is needed. It has to be recognised, however, that where the constituents of plants are liable to undergo chemical change with drastic treatment, each case must be considered individually in order to avoid risk of loss of toxicity.

The results, which are the averages of a number of experiments, are expressed in Diagram 1 and Table I.

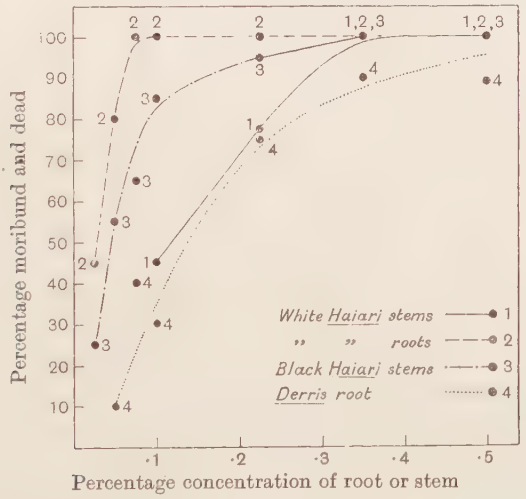


Diagram 1. Showing toxicities of alcoholic extracts of Haiari and Derris.

[1] A preliminary reference to the high toxicity of these plants was made in a letter to *Nature*, 1925, cxvi, 243.

Table I.

Toxicity to A. rumicis *of Black and White Haiari and Derris.*

[N = not affected. S = slightly affected. M = moribund. D = dead.]

Material	Concentration: Percentage of dried extract	Concentration: Equivalent percentage of plant	No. of tests	N %	S %	M %	D %	M & D %
Black Haiari (stems)	—	5·0–0·5	11	—	—	—	—	100
Alcoholic extract	0·023	0·35	2	—	—	20	80	100
	0·016–0·013	0·25–0·2	4	—	5	42·5	52·5	95
	0·006	0·1	2	10	5	50	35	85
	0·005	0·075	2	25	10	40	25	65
	0·0033	0·05	2	30	15	20	35	55
	0·0016	0·025	2	70	5	15	10	25
Black Haiari (stems) *Aqueous extract*	Not completely toxic at 5 % of plant							
White Haiari (stems)	—	5·0–0·5	11	—	—	—	—	100
Alcoholic extract	0·027	0·35	2	—	—	25	75	100
	0·02–0·0156	0·25–0·2	4	5	17·5	37·5	40	77·5
	0·0078	0·1	2	15	40	15	30	45
	Below 0·1 % results rather irregular							
White Haiari (stems)	—	5·0	1	10	40	20	30	50
Aqueous extract	—	3·5	1	40	10	20	30	50
	—	2·0	1	100	—	—	—	—
White Haiari (roots)	—	5·0–0·35	12	—	—	—	—	100
Alcoholic extract	0·02–0·017	0·25–0·2	4	—	—	30	70	100
	0·0087	0·1	2	—	—	20	80	100
	0·0065	0·075	2	—	—	55	45	100
	0·0043	0·05	2	—	20	45	35	80
	0·0021	0·025	2	35	20	15	30	45
White Haiari (roots)	—	5·0	1	—	—	60	40	100
Aqueous extract	—	3·5	1	—	—	90	10	100
	—	2·0	1	10	—	60	30	90
	—	1·0	1	20	—	80	—	80
	—	0·5	1	60	30	10	—	10
	—	0·25	1	100	—	—	—	0
Derris elliptica	—	2·0–1·0	2	—	—	—	—	100
Alcoholic extract	—	0·5	2	11	—	61	18	89
	—	0·35	1	—	10	90	—	90
	—	0·25–0·2	2	10	15	60	15	75
	—	0·1	1	40	30	30	—	30
	—	0·075	1	40	20	40	—	40
	—	0·05	1	70	20	10	—	10

It will be observed from this table and diagram that the roots of White Haiari are slightly more toxic than the stems of this plant or than the stems of Black Haiari and that all three have a somewhat greater insecticidal value than a sample of *Derris* tested at the same time. Too much stress must not be laid on these differences as, although probably valid, it is possible that the amounts of poison present may depend upon the age of the plants and thus render any arrangement in order of toxicities true only of the particular samples tested. The roots and stems

of White Haiari and the stems of Black Haiari undoubtedly have insecticidal values of a high order[1].

Preliminary practical trials on aphis-infested bean plots indicated that alcoholic extracts of these plants applied at a concentration equivalent to 1 per cent. of root and stem (the only strength tested), with 0·25 per cent. soft soap as a spreader and wetter, secured almost complete control.

Table II.

Toxicity to A. rumicis *of products isolated from Black and White Haiari and Derris.*

[N = not affected. S = slightly affected. M = moribund. D = dead.]

(Figures for nicotine are included for comparison.)

Material	Concentration in gm. per 100 c.c.	No. of tests	N %	S %	M %	D %	M & D %
Colourless crystals from stems of Black Haiari	0·1	1	—	—	20	80	100
	0·01	2	—	—	40	60	100
	0·0075	1	—	10	20	70	90
	0·005	2	5	5	60	30	90
	0·0035	1	30	—	70	—	70
	0·002	1	80	—	20	—	20
Resin from stems of Black Haiari	0·1	1	—	—	20	80	100
	0·05	1	—	10	20	70	90
	0·025	1	—	20	80	—	80
	0·01	1	—	50	40	10	50
	0·005	—	70	10	10	10	20
Colourless crystals from roots of White Haiari	0·01	2	—	—	80	20	100
	0·0075	2	—	15	70	15	85
	0·005	2	3·3	26·7	66·7	3·3	70
	0·0035	2	30	35	35	—	35
	0·002	2	35	30	35	—	35
	0·001	2	86·7	6·7	3·3	3·3	6·6
Resin from roots of White Haiari	0·1–0·025	3	—	—	—	—	100
	0·01	1	—	20	20	60	80
	0·005	1	30	40	20	10	30
	0·0035	1	40	50	10	—	10
Tubatoxin from Derris	0·25–·0075	9	—	—	—	—	100
	0·005	1	10	—	90	—	90
	0·0035	1	70	—	30	—	30
	0·002	1	70	10	20	—	20
	0·001	1	70	10	20	—	20
Nicotine	0·08	—	—	—	—	—	98
	0·06	—	—	—	—	—	92
	0·04	—	—	—	—	—	60
	0·02	—	—	—	—	—	25·5
	0·01	—	—	—	—	—	6
	0·005	—	—	—	—	—	6·5
	0·0025	—	—	—	—	—	2

[1] Since sending this paper to the press, we have been able to test the *roots* of Black Haiari; they were found to be about as toxic as the stems. The leaves of both Haiaris were not toxic at any concentration tested.

Table II shows the results obtained with some of the constituents isolated from these plants. They are expressed diagrammatically in Diagram 2.

When alcoholic extracts of Haiari were concentrated, crystals separated from the solutions; these were filtered off and recrystallised twice. Their melting-point was between 160·5 and 161·5° C.; this is within one or two degrees of the melting-point of tubatoxin, shown by Fryer, Stenton, Tattersfield and Roach (7) to be one of the main toxic constituents of *Derris elliptica*. The toxicity of these crystals was therefore compared with that of tubatoxin. This we were able to do through the kindness of Dr H. McCombie who placed at our disposal a sample of pure tubatoxin prepared from *Derris*. Table II and Diagram 2 indicate that the insecticidal properties of the colourless crystals isolated from the roots of White Haiari and the stems of Black Haiari are almost identical with those of tubatoxin, and that one curve will represent with considerable accuracy the whole range of toxicity for all three. As the crop of crystals from White Haiari roots was the most abundant, they were purified by further recrystallisation, the melting-point of the pure product being 162·5–163·5° C., agreeing with that of tubatoxin. The crystals, which contain no nitrogen, are either plates or needles and give

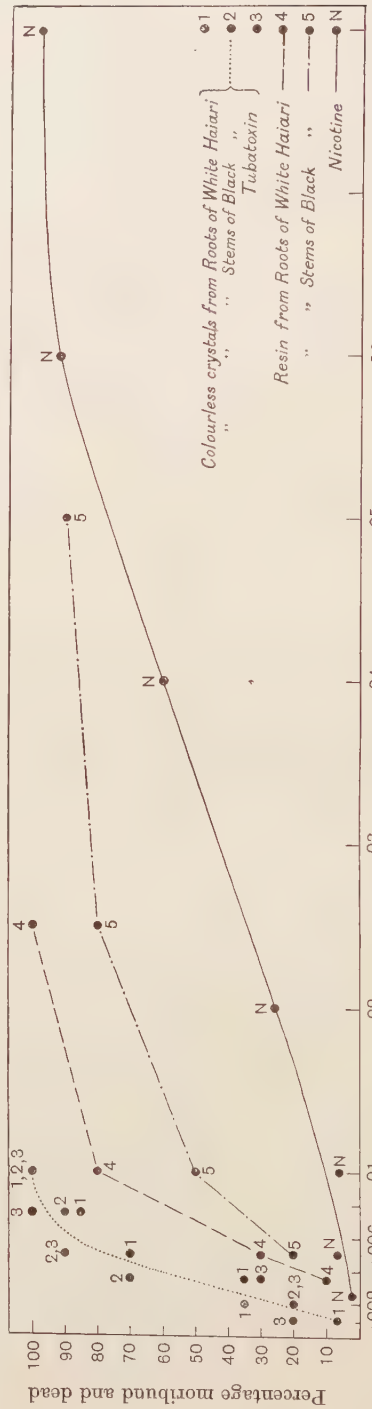

Colourless crystals from Roots of White Haiari
" " Stems of Black "
Tubatoxin
Resin from Roots of White Haiari
" " Stems of Black "
Nicotine
Percentage moribund and dead
Concentration in gm. per 100 c.c.

Diagram 2. Showing toxicities of products isolated from Haiari and Derris (with nicotine for comparison)

Durham's colour test (nitric acid followed by ammonia[1]); they also give rise to yellow needles when boiled with or allowed to stand with alcohol, in all these points agreeing with the behaviour of tubatoxin derived from *Derris*. Dr McCombie, who kindly undertook a further examination of the crystals, established their identity with tubatoxin by means of a mixed melting-point determination.

It is evident therefore that tubatoxin is the main insecticidal constituent of these plants and, as it is present in fairly considerable quantities, they may prove a very suitable source of that compound.

Diagram 2 shows that the toxicity of these crystals is of a distinctly higher order than that of nicotine when tested in our apparatus. It would be rash however to deduce from this that the difference would be equally marked when applied on a field scale. Tubatoxin is non-volatile and solid and a direct hit by finely suspended or dissolved particles is probably essential; whereas nicotine being slightly volatile may have in the vapour phase a lethal effect at a distance from the spot where the liquid reached the plant.

The figures for the resins are also shown in Table II and Diagram 2. The resins were isolated by precipitating the gums, etc., from alcoholic extracts by means of basic lead acetate, filtering, precipitating the lead in the filtrate with sulphuretted hydrogen, freeing from the latter by a current of air, and distilling off the volatile solvents *in vacuo*. The residue was allowed to stand with methylated ether in which it was only partially soluble; the ether solution was decanted and precipitated with petroleum ether, the residue being taken up again and reprecipitated. It was not found possible to free the resins entirely from crystalline matter and therefore the toxicities shown are probably higher than the true values. It is clear however that the resins are not so toxic as the crystals.

Preliminary trials were made with the roots of White Haiari and the stems of Black Haiari as *stomach poisons* against larvae of *Selenia tetralunaria* and *Abraxas grossulariata*. Foliage was sprayed with alcoholic extracts diluted to be equivalent to 1 per cent. of the respective plants. There was definite evidence for toxicity to the *Selenia* larvae, which was not so marked in the case of the larvae of *Abraxas*. The most noteworthy feature was that the foliage sprayed by these extracts was rendered strongly repellent to both species; there was no visible amount of feeding.

[1] See Tattersfield, F. and Roach, W. A., The Chemical Properties of *Derris elliptica*, *Ann. App. Biol.* 1923, x, p. 5.

Table III.

Toxicity of alcoholic extracts of three species of Tephrosia *to* Aphis rumicis.

[N = not affected. S = slightly affected. M = moribund. D = dead.]

Material	Concentration percentage of plant	No. of tests	N %	S %	M %	D %	M & D %
T. toxicaria (roots)	5·0–0·5	9	—	—	—	—	100
	0·35	2	5	—	75	20	95
	0·25–0·2	3	6	—	71	23	94
	0·1	2	5	5	65	25	90
	0·075	1	50	20	10	20	30
	0·05	2	50	25	20	5	25
T. toxicaria (leaves)	Not appreciably toxic at 5 % of leaf						
T. toxicaria (stems)	5·0	1	30	—	70	—	70
	3·5	1	30	20	40	10	50
	2·0	1	70	20	10	—	10
	1·0	1	80	10	10	—	10
T. Vogelii (leaves) (new sample)	2·0–1·0	6	—	—	—	—	100
	0·75	3	—	—	37	63	100
	0·5	3	—	3	60	37	97
	0·35	1	10	—	30	60	90
	0·25–0·2	3	7	18	58	17	75
	0·1	1	10	10	60	20	80 (?)
	0·05	1	60	30	10	—	10
T. Vogelii (stems)	5·0	1	—	—	—	100	100
	3·5	1	—	—	—	100	100
	2·0	1	—	—	30	70	100
	1·0	1	—	—	60	40	100
	0·75	1	—	20	60	20	80
	0·5	1	—	30	70	—	70
T. Vogelii (seeds) (sample from the Sudan)	2·0	1	—	—	30	70	100
	1·5	1	—	—	60	40	100
	1·0	1	30	10	—	60	60
	0·75	1	60	30	—	10	10
	0·5	1	80	20	—	—	0
	0·25	1	100	—	—	—	0
T. Vogelii (roots and stems)	5·0	1	—	10	80	10	90
	3·5	1	10	—	90	—	90
	2·0	1	20	10	50	20	70
	1·0	1	30	70	—	—	0
T. candida (roots)	7·5	1	—	—	40	60	100
	5·0	1	10	10	50	30	80
	3·5	1	—	10	70	20	90
	2·0	2	12·5	18·75	43·75	25	68·75
	1·5	1	30	10	40	20	60
	1·0	2	80	5	5	10	15
	0·75	1	90	—	—	10	10
	0·5	1	90	10	—	—	0
T. candida (stems)	7·5–5·0	3	—	—	—	—	100
	3·5	1	—	—	100	—	100
	2·0	2	15	10	60	15	75
	1·0	2	30	15	30	25	55
	0·75	1	60	20	—	20	20
	0·5	1	60	20	20	—	20

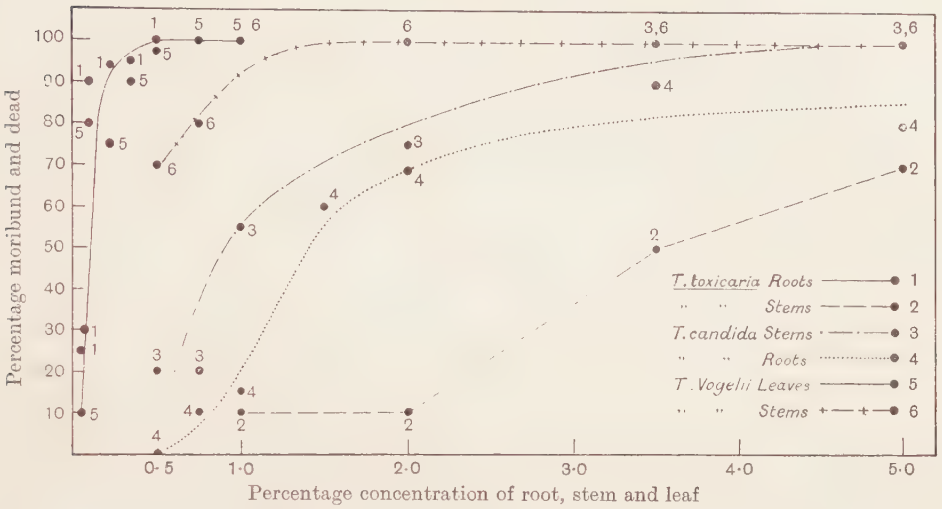


Diagram 3. Showing toxicity of alcoholic extracts of 3 species of *Tephrosia*.

(B)[1] *Genus* Tephrosia. Since the publication of our paper on *Tephrosia Vogelii* we have received a fresh consignment of this plant from the Sudan; we have also had samples of *T. toxicaria* from British Guiana and of the roots and stems of *T. candida* from the West Indies. The latter, so far as we know, is not used as fish poison.

The experimental values are set out in Table III and Diagram 3. For the determination of these values alcoholic extracts were prepared as in the case of Haiari (see p. 427); they were not taken to dryness but diluted directly to the required concentrations, control experiments being carried out in the usual manner.

Inspection of Table III shows that with *T. Vogelii* the leaves and seeds are more toxic than the stems, in agreement with previous results(3); and although only very small amounts of the roots, to some extent mixed with portions of stem, have been available, it would appear that they do not possess a toxicity of a high order. On the other hand, the roots of *T. toxicaria* are more toxic than the stems, while the leaves possess insecticidal properties only in a very slight degree. The roots and stems of *T. candida* are much less toxic than the roots of *T. toxicaria* but are slightly more so than the stems and leaves of the latter species. The leaves of *T. candida* were not available. With both *Vogelii* and

[1] A short account of the distribution of species of *Tephrosia* and allied genera, and of their use as fish poisons has recently been published by A. Chevalier (*Compt. Rend.* 1925, CLIII, 1520).

toxicaria, the parts of the plant which are most potent as contact insecticides are the parts which are used by the natives as fish poisons.

T. candida so far as tested by us does not show more than very moderate insecticidal properties. The roots of *T. toxicaria*, on the other hand, are highly toxic to aphides and promise to be as well worth further investigation as the roots and stems of Haiari and the leaves and seeds of *T. Vogelii*, and it is hoped to make trials on a larger scale.

In our previous paper on the toxicity of *Tephrosia Vogelii*, we noted that it seemed probable that the compound tephrosin isolated by Hanriot (8) from this plant and also studied at the Imperial Institute (9), and shown to be the constituent mainly concerned in its action as a fish poison, was also responsible for its toxicity to aphides.

Following Hanriot's method with slight modifications, and without attempting quantitative work, we have isolated from the leaves of *Tephrosia Vogelii*, a resin and two colourless crystalline products melting at 191–193° C. and 201·5° C. respectively. In addition, we obtained an oil apparently identical with Hanriot's "tephrosal," and a more viscous oil probably containing traces of "tephrosal," a very small quantity of a colourless crystalline derivative melting between 120° and 130° C., and batches of yellow needles which were evidently a reaction product formed during the process of extraction. The two lots of crystals melting at 191–193° C. and 201·5° C. were very similar in appearance and properties, both having a tendency to give rise to yellow needles on boiling with alcohol or acetone. They differed in that the former gave Durham's reaction (see p. 431), whereas the latter did not. The strength of caustic potash used in Hanriot's method for rendering the resin insoluble, appeared to determine which of these two products was obtained, suggesting that they do not occur as such in the plant. The crystals melting at 191–193° C. could be separated by fractional crystallisation into two products only, one melting at 190–191° C. and the other at 192–193·5° C.; no yield of crystals was obtained melting at 201·5° C. They did not appear to be identical with Hanriot's tephrosin, the melting-point of which he gave as 187° C., but they are probably the same as the product described in the *Imp. Inst. Bull.* (9) which melted at 192° C. Owing to the drastic treatment employed in the process of isolation, it is open to doubt whether any of these compounds, including Hanriot's tephrosin, are present as such in the plant.

We have carried out a series of tests with the various derivatives referred to and the results are given in Table IV and Diagram 4. The data obtained with the crystalline products were rather irregular and

Table IV.

Toxicity to A. rumicis *of products derived from* Tephrosia toxicaria *and* Vogelii.

[N = not affected. S = slightly affected. M = moribund. D = dead.]

Material	Concentration in gm. per 100 c.c.	No. of tests	N %	S %	M %	D %	M & D %
T. toxicaria. Resin from roots (soluble in ether, pptd. by petroleum ether)	0·2–0·05	4	—	—	—	—	100
	0·025	1	—	—	100	—	100
	0·01	1	40	50	10	—	10
T. toxicaria. Resin from roots (soluble in a mixture of ether and petroleum ether)	0·2–0·075	3	—	—	—	—	100
	0·05	1	—	—	60	40	100
	0·025	1	—	10	80	10	90
	0·01	1	10	—	90	—	90
	0·0075	1	60	30	10	—	10
T. Vogelii. Tephrosal from leaves	1·0	1	—	—	—	100	100
	0·5	1	50	30	10	10	20
T. Vogelii. Oil from leaves	1·0	1	20	—	20	60	80
	0·5	1	20	—	40	40	80
	0·2	1	30	30	10	30	40
	0·1	1	80	—	20	—	20
T. Vogelii. Resin from leaves	1·0	1	—	—	10	90	100
	0·5	1	—	—	40	60	100
	0·2	1	—	10	60	30	90
	0·1	1	—	10	50	40	90
	0·05	1	20	—	70	10	80
	0·025	1	10	40	40	10	50
	0·01	1	80	20	—	—	0

Yellow needles (M.P. 213° C.) from roots of *T. toxicaria*, and crystalline derivatives from the leaves of *T. Vogelii* melting at 123° C., 184–187° C., 190–191° C., 192–193° C., 192° C., 201–201·5° C., 227° C. were not appreciably toxic at or below 0·2 % concentration. (The crystals of M.P. 227° C. were yellow needles.)

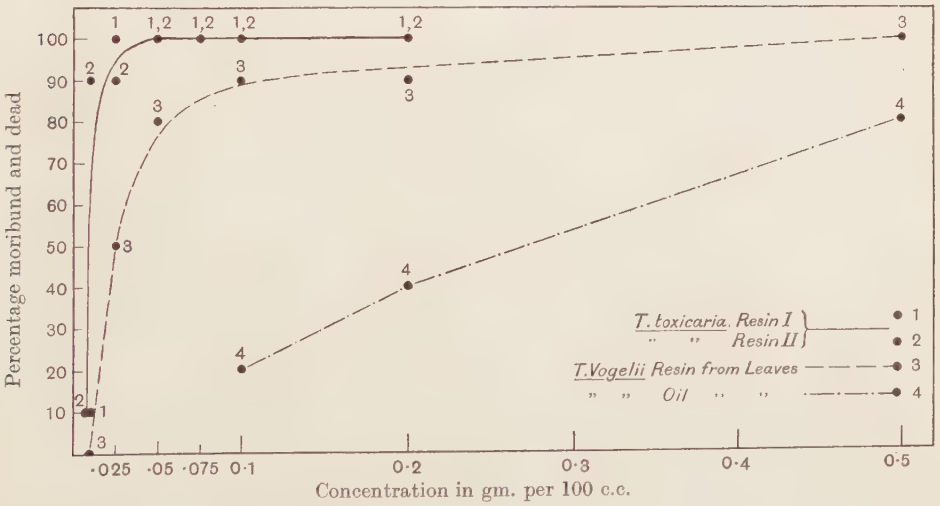


Diagram 4. Showing toxicities of products derived from *T. toxicaria* and *Vogelii*.

they did not show a very high degree of toxicity to *A. rumicis*. Owing to the relative insolubility of these bodies in organic solvents, considerable difficulties were met with in making up fluids for spraying and it is possible that the toxicity was actually greater than that indicated. It is improbable, however, that they are the chief toxic constituents of the leaves of *T. Vogelii*.

The resin isolated was highly toxic but our experiments with it are hardly sufficiently comprehensive to warrant an opinion as to whether or not this body is mainly responsible for the toxicity of the plant.

A preliminary attempt was also made to separate the toxic constituents in the roots of *T. toxicaria*, using the basic lead acetate method. A resinous material isolated proved highly toxic to *Aphis rumicis*, but the only other product obtained in sufficient quantity to test—a compound crystallising in yellow needles melting at 213–214° C.—showed no toxic properties at the concentrations tried.

Preliminary trials with alcoholic extracts of the leaves of *T. Vogelii* and the roots of *T. toxicaria* as *stomach poisons* gave results very similar to those given by White and Black Haiari (see p. 431).

Other Plants.

(A) *Genus* Lupinus. The tropical plants just dealt with all belong to the Leguminosae and it was thought of interest to test the insecticidal properties of some members of this order occurring in this country. The Lupins, being readily available and having toxic properties, suggested themselves.

The leaves, stems and roots of three species were tested but were not found to possess any marked poisonous action.

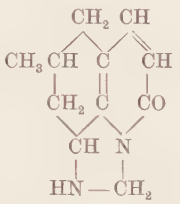
Alcoholic extracts of the seed of Perennial and an annual Blue Lupin were however found to have a certain toxicity; the Perennial Lupin appeared, in a preliminary set of trials, to be rather more effective than the Blue Lupin. The work is not far enough advanced to enable us to draw definite conclusions, the tests indicating that toxicity might be affected by the after-treatment of the crude extracts and by the degree of purification. The toxicity of these plants is not in any case of the same order as that of those dealt with above.

(B) *Cytisine and Cytisine-containing plants.* Another group of Leguminous plants which seem likely to be of interest are those which contain the poisonous alkaloid cytisine. This alkaloid is fairly widely distributed in nature, being found in many species of *Cytisus*[1] and also in several

[1] An infusion of broom tops has been recommended as a larvicide. (See *Pharm. Journ. and Pharmacist*, 1917, **44**, 139.)

species of *Genista*, *Ulex*, *Sophora* and *Baptisia*. It has been isolated from the seeds of Laburnum (1·56 per cent.), Gorse (1·03 per cent.) and Broom. Von Plugge(10) states the cytisine content of *Sophora speciosa* Benth., the Poison Bean of Mexico and Texas as 3·23 per cent. and of the seeds of *Sophora secundiflora* Lagasca, a small shrub of Texas, as 3·47 per cent. The seeds of *Sophora tomentosa* Schrurbaum are said to contain 2·06 per cent. from which plant it has also been isolated from the leaves, and the cytisine content of *Baptisia australis* is given as 1·56 per cent.

The alkaloid was apparently first prepared and named by Gray and isolated in a pure state by Husemann and Marmé, who ascribed to it the formula $C_{11}H_{14}ON_2$. Its constitution has been partially elucidated by the work of Ewins(11) and of Späth(12) and other investigators. According to Späth the most probable structure is



Dale and Laidlaw(13) in their important paper on the physiological action of cytisine pointed out the close similarity in physiological action of cytisine and nicotine and that quantitatively in this respect they are scarcely distinguishable.

In view of these conclusions it appeared desirable to test the insecticidal action of this alkaloid and of extracts of the seeds in which it occurs. By the kindness of Dr H. H. Dale, who placed at our disposal a considerable quantity of the crude alkaloid, we were able to carry out a number of quantitative determinations of its toxicity to *Aphis rumicis*, and to the eggs of the moth *Selenia tetralunaria*. Unlike nicotine, cytisine did not prove toxic to these insect eggs. The results obtained on *A. rumicis* are set out in Table V and Diagram 5.

Reference to this table and diagram shows that whereas the crude and pure samples of cytisine have an insecticidal value of a fairly high order, neither is so toxic as nicotine. The crude sample, as would be expected, is somewhat less toxic than the pure.

In expressing the results obtained with Gorse, Laburnum and *Sophora tomentosa*, we have made comparisons on the basis of the percentage concentrations of the dried extracts of the seed. One curve fits approximately the whole of the points for the first three; while the

Table V.

Toxicity of cytisine and cytisine-containing plants, lobeline and nicotine to Aphis rumicis.

[N = not affected. S = slightly affected. M = moribund. D = dead.]

Material	Concentration in gm. per 100 c.c.	No. of tests	N %	S %	M %	D %	M & D %
Cytisine (pure)	1·0–0·5	3	—	—	—	100	100
	0·35	1	—	—	30	70	100
	0·2	1	20	—	20	60	80
	0·1	1	10	30	30	30	60
	0·05	1	90	—	10	—	10
Cytisine (crude)	1·0–0·75	7	—	—	—	—	100
	0·5	5	2	—	43	55	98
	0·4	2	20	—	45	35	80
	0·35–0·3	4	22·5	—	32·5	45	77·5
	0·25–0·2	5	27·7	12·7	23·4	36·2	59·6
	0·1	5	60	8	4	28	32
	0·06–0·04	6	88	3·4	1·7	6·9	8·6
Broom seeds (crude chloroform extract)	5·0	1	—	—	30	70	100
	2·5	1	—	—	70	30	100
	1·0	1	60	—	20	20	40
	0·5	1	90	—	—	10	10
Laburnum seeds (crude chloroform extract)	5·0	1	—	—	20	80	100
	2·5	1	10	10	50	30	80
	1·0	1	40	10	20	20	40
	0·5	1	80	—	—	20	20
Gorse seeds (crude alcohol extract)	10·0–3·0	3	—	—	—	100	100
	2·0	2	—	—	5	95	100
	1·0	2	35	10	25	30	55
	0·5	1	77·8	—	—	22·2	22·2
Lobeline	1·0	1	—	—	60	40	100
	0·75	1	—	—	60	40	100
	0·5	1	—	—	100	—	100
	0·25	1	30	—	70	—	70
	0·1	1	80	10	10	—	10
	0·05	1	80	20	—	—	0
Nicotine (the same data as in Table II)	0·08	—	—	—	—	—	98
	0·06	—	—	—	—	—	92
	0·04	—	—	—	—	—	60
	0·02	—	—	—	—	—	25·5
	0·01	—	—	—	—	—	6
	0·005	—	—	—	—	—	6·5
	0·0025	—	—	—	—	—	2
Alcohol controls	100	—	100	—	—	—	0
	50	—	100	—	—	—	0
	25	—	90	10	—	—	0
	10	—	80	20	—	—	0

Crude chloroform extract of *Sophora* seeds—not materially toxic, at a concentration of 5 % of dried extract.

Crude alcohol extracts of the combined seeds and pods of Gorse and Broom—not materially toxic at a concentration of 10 % of dried extract.

Alcohol extracts of leaves and seeds of *Lobelia*—not materially toxic at a concentration of 5 % of dried extract.

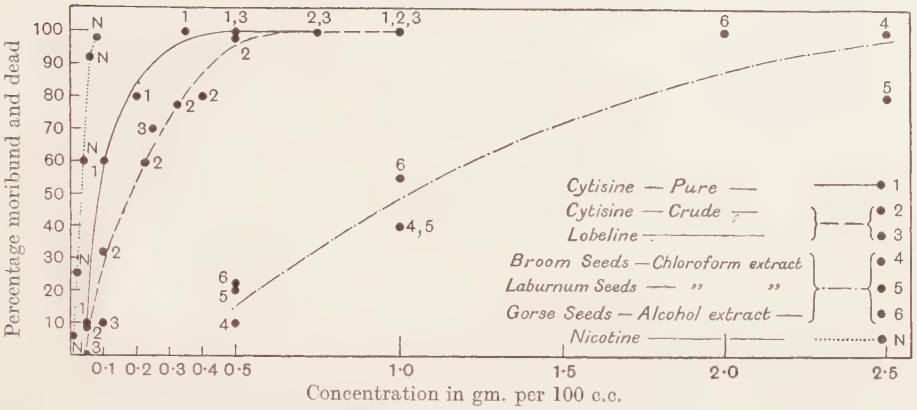


Diagram 5. Showing toxicities of cytisine, cytisine containing plants, lobeline and nicotine.

seeds of *Sophora tomentosa* showed little toxic action, a rather unexpected result, possibly due to the seeds having been taken at a time when the cytisine content was not at a maximum.

The alcohol extractions were carried out in Soxhlet's apparatus and the percentage of dry extract obtained was as follows:

Broom seeds (*Cytisus scoparius*)	10·64 %
Broom seeds and pods	5·68
Gorse seeds (*Ulex europaeus*)	16·74
Gorse seeds and pods	6·49
Laburnum seeds (*Cytisus laburnum* L.)	20·09

The conclusion to be drawn from these experiments is that crude extracts of cytisine-containing seeds, with the possible exception of laburnum, are not likely to prove of practical importance. Cytisine itself, if it could be prepared comparatively cheaply, would be worth further consideration. Cytisine showed no action as a stomach poison against larvae of *Selenia tetralunaria*.

(C) *Lobeline and* Lobelia. *Lobelia inflata* contains the alkaloid lobeline which is stated by Dale and Laidlaw (13), Edmunds (14) and Dreser (15) to possess in its physiological effects a close resemblance to nicotine.

The insecticidal values obtained with lobeline are stated in Table V and Diagram 5.

Owing to the sternutative properties shown by this compound it does not offer much prospect for practical use and the values expressed in this paper are based upon a few tests only. These showed that pure lobeline had a toxicity about equal to that of crude cytisine, while extracts of *Lobelia* proved only slightly toxic.

(D) *Mammea americana* from the West Indies, and *Asclepias curassavica* from British Guiana, among other plants, were tested and not found sufficiently toxic to warrant further investigation. Alcoholic extracts of the stems and roots of "Conami" from British Guiana were ineffective at concentrations equivalent to 5 per cent. of the plant, while the flowers had a slight insecticidal action at this concentration.

We also tested alcoholic extracts of Tomato (leaves and stems), Camomile (flowers, leaves and stems), Feverfew and Tansy. Those plants have all been recorded on various occasions (see McIndoo and Sievers(2)) as having definite insecticidal properties or as being repellent to insects, but they showed no significant toxicity to *Aphis rumicis* under our experimental conditions. Similar results were obtained with commercially prepared extracts of Belladonna, Hellebore, *Cannabis Indica*, Stramonium, Colocynth, Hyoscyamus and Digitalis.

Miscellaneous Alkaloids.

The results obtained with a certain number of alkaloids are set out in Table VI and Diagram 6.

Arecoline, $C_{18}H_{13}NO_2$, is one of the alkaloids of Areca or Betel Nut, in which it is said to occur to the extent of 0·07–0·1 per cent. The pure alkaloid is not completely toxic to *A. rumicis* below 0·5 per cent.

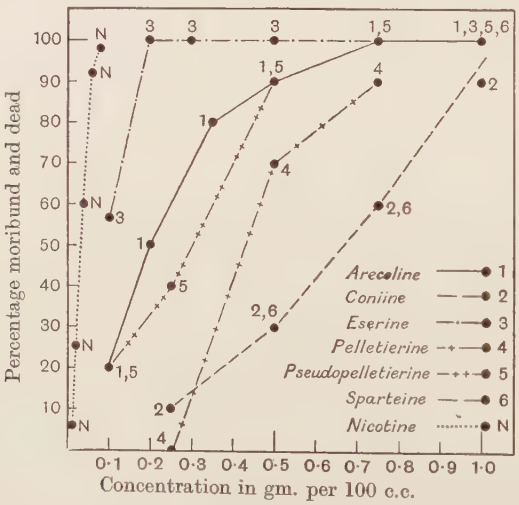


Diagram 6. Showing toxicities of certain alkaloids.

Table VI.

Toxicity of certain alkaloids to Aphis rumicis.

[N = not affected. S = slightly affected. M = moribund. D = dead.]

Material	Concentration in gm. per 100 c.c.	No. of tests	N %	S %	M %	D %	M & D %
Arecoline (hydrochloride)	0·6 base	1	—	—	40	60	100
	0·4	1	20	30	30	20	50
	0·2	1	70	—	20	10	30
Arecoline (base)	1·0	1	—	—	—	100	100
	0·75	1	—	—	20	80	100
	0·5	1	—	10	70	20	90
	0·35	1	10	10	40	40	80
	0·2	1	40	10	10	40	50
	0·1	1	70	10	—	20	20
Coniine	1·0	1	10	—	30	60	90
	0·75	1	40	—	20	40	60
	0·5	1	70	—	10	20	30
	0·25	1	90	—	—	10	10
Eserine	1·0	1	—	—	—	100	100
	0·5	1	—	—	—	100	100
	0·3	1	—	—	—	100	100
	0·2	1	—	—	—	100	100
	0·1	3	30	13·3	6·6	50	56·6
Pelletierine	0·75	1	—	10	50	40	90
	0·5	1	10	20	50	20	70
	0·25	1	70	30	—	—	0
Pseudopelletierine	1·0	1	—	—	—	100	100
	0·75	1	—	—	60	40	100
	0·5	1	—	10	—	90	90
	0·25	1	50	10	20	20	40
	0·1	1	70	10	—	20	20
Sparteine (base)	1·0	1	—	—	30	70	100
	0·75	1	40	—	10	50	60
	0·5	1	70	—	—	30	30

Hydrastine, Hydrastinine, Hordenine methiodide and Narcotine were not materially toxic at or below a concentration of 1 gm. per 100 c.c.

Coniine, $C_8H_{17}N$, is found in the seeds of *Conium maculatum*. It is a very powerful poison to higher animals (16). To insects, however, when used as a contact poison its toxicity is surprisingly low.

Eserine (*Physostigmine*), $C_{15}H_{21}N_3O_2$, is the alkaloid of Calabar Beans. Its constitution is not known but a considerable amount of information is available about some of its decomposition products.

Eserine is very poisonous to higher animals, having a direct paralytic action upon the central nervous system. Underhill (17) states that in some respects its physiological activity is similar to that of nicotine.

This alkaloid was tested by taking up in a small quantity of benzene and emulsifying in 1 per cent. saponin solution. It is almost completely

toxic to *A. rumicis* at concentrations between 0·1 and 0·2 per cent., and approaches the toxicity of nicotine.

Hydrastine, *Hydrastinine* and *Narcotine* were tested but possess no interest whatever from an insecticidal point of view.

Pelletierine proved toxic at a concentration of 0·75 per cent. and *Pseudo-pelletierine* at a concentration of 0·5 per cent.

Sparteine, $C_{15}H_{26}N_2$, is found in Broom. It possesses a narcotic action reminiscent of coniine and to some extent of nicotine (Abderhalden, *Handlexikon*, 1911, v, 115).

Sparteine was tested both as sulphate and as base. The sulphate was not found materially toxic at a concentration equal to 1 per cent. of the base; the base itself, however, at this concentration gave 100 per cent. control and at 0·75 per cent. a control of 60 per cent. The toxicity is only of the same order as that of coniine.

Discussion and Conclusions.

In preceding sections a detailed account is given of laboratory experiments on the toxicity to *Aphis rumicis* of a number of plant extracts and plant derivatives. These include (1) a group of plants which show marked poisonous action to fish; (2) certain alkaloids which are known to have a physiological action on the higher animals closely similar to that of nicotine, and extracts of the plants from which they are derived; (3) a few other alkaloids.

(1) Amongst the plants used as fish poisons, showing a high toxicity under our experimental conditions, were the roots and stems of White Haiari, the stems of Black Haiari (species of *Lonchocarpus*), the roots of *Tephrosia toxicaria*, the leaves of *T. Vogelii*. It is a matter of interest that all these plants are of the order Leguminosae. The alcoholic extracts are highly toxic, distinctly more so than nicotine, weight for weight, and are of almost the same order as that of the toxic derivatives isolated in a pure state from the plants.

Of the compounds isolated from extracts of these plants those found most effective from an insecticidal point of view, were in all cases non-volatile solids. From the Haiari plants the poisons obtained were: (*a*) colourless crystals melting at 162·5–163·5° C. and identical with tubatoxin, the crystalline poisonous constituent of *Derris elliptica*, and (*b*) a resin which proved less toxic. Tubatoxin and the resins were found to be several times more poisonous to *A. rumicis* than nicotine.

From *T. Vogelii* a number of derivatives were isolated, the most important being a resin (highly poisonous) and colourless crystals

melting at 191–193° C., and 201·5° C. respectively, with which indefinite results were obtained.

The colourless crystals possessed very similar properties to Hanriot's tephrosin, but their toxicity was much less than would be expected in view of the results obtained with the alcoholic extracts of the leaf. In the case of solid insecticides, size of particle in the spray fluid is of great importance, and owing to the comparative insolubility of these crystals in most solvents, it was difficult to prepare a really satisfactory suspension. Although finely dispersed, discrete particles easily distinguishable under the microscope were present, whereas the spray fluids prepared direct from alcoholic extracts of the leaf were colloidal in type. Further work of a quantitative nature is needed to make clear the significance of particle size in this connection.

When tested as a stomach poison, extracts of Haiari and *Tephrosia Vogelii* and *toxicaria* showed both a toxic and a repellent action to the larvae of the insects used in our tests (*S. tetralunaria* and *A. grossulariata*).

(2) Of the alkaloids known to have a physiological action closely akin to that of nicotine the two most important are cytisine and lobeline. The researches of Dale and his co-workers have demonstrated the close similarity in this respect of these alkaloids. Our experiments show that both cytisine and lobeline have moderately high values as contact insecticides, but that neither is so toxic as nicotine. Cytisine, unlike nicotine, showed little toxic action to insect eggs or as a stomach poison to biting insects.

(3) A few other alkaloids were included in our tests. Some of these have been considered by various investigators to have, in some of their physiological effects, a similarity to nicotine. Only eserine approached nicotine in the intensity of its toxic action, while coniine and sparteine showed a surprisingly feeble toxicity.

The greater part of our data applies to experiments on a laboratory scale; in certain cases, however, preliminary larger scale trials have been carried out. In general there is a close agreement with the results of the laboratory tests, but in some cases irregularities were noted and new problems undoubtedly arise in transferring the investigation to the larger field. A *sine qua non* of quantitative laboratory trials is that a maximum of efficiency should be obtained and that each insect should receive the same dose of poison under precisely controlled conditions. This is obviously impossible of attainment in the field. In the case of the fish poisons, where the toxic constituents are solid and non-volatile

at ordinary temperatures, questions of dispersion of the poison in the spray fluid, of the fineness of the spray and the pressure at which it impinges on the insect are all of importance and have not yet been investigated.

The number of plants actually employed as insecticides at the present time in any quantity is very small; probably Tobacco, Pyrethrum, Hellebore and Derris almost exhaust the list. Toxicity to insects is the first but by no means the only essential requirement for a plant insecticide. Abundant growth in the wild state or comparative ease of cultivation and a minimum of harmfulness to plants and to human beings are very important considerations. Our information with regard to the Haiari plants is not sufficient to enable us to express an opinion as to how readily a supply could be obtained; but in the case of the *Tephrosia* species, *Vogelii* certainly and *toxicaria* probably, could be obtained in bulk with comparative ease.

As we have already pointed out, much remains to be done in the way of large-scale experiments on the degree of control obtainable in the field with these materials; and further information is required with regard to the best means of preparing an extract for spraying, and of securing efficient spreading, wetting and adherence. We desire at the moment to draw attention to the very high toxicity to aphides exhibited by extracts of these plants and by certain substances isolated from them. If the difficulties that still lie in the way of successful application in practice can be overcome, there is here a supply of material, harmless to foliage and of quite outstanding toxicity as a contact poison to insects.

Summary.

1. An account is given of laboratory experiments on the toxicity to *Aphis rumicis* L. of extracts of a considerable number of plants, including some tropical fish-poisons, Lupins, Broom, Gorse, Lobelia and others.

2. Alcoholic extracts of certain tropical plants used as fish-poisons are shown to have a high toxicity under the conditions of the experiments. The roots and stems of White Haiari, and the stems of Black Haiari (both species of *Lonchocarpus* from British Guiana), the roots of *Tephrosia toxicaria* and the leaves of *T. Vogelii* all possess notable insecticidal properties. The roots and stems of *T. candida* are less toxic.

3. Preliminary experiments indicate that the Haiaris and *T. Vogelii* and *T. toxicaria*, when tested as stomach poisons, exert both a repellent and toxic action to caterpillars.

4. Certain derivatives isolated from these plants were tested. The most toxic substance obtained from the Haiaris is shown to be identical with tubatoxin, the crystalline poison found in *Derris elliptica*. Tubatoxin proved to be several times more toxic than nicotine. In the case of *Tephrosia Vogelii* and *T. toxicaria*, the most toxic substances isolated were resinous in nature. Crystals closely corresponding to tephrosin, as isolated by Hanriot, were less toxic.

5. A number of alkaloids was also investigated. Cytisine and lobeline, known to have a physiological action on higher animals similar to that of nicotine, were found somewhat less toxic than nicotine to aphides. Eserine was the only other alkaloid tested which approached nicotine in toxicity.

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A QUANTITATIVE EXAMINATION OF THE TOXICITY OF 3:5-DINITRO-O-CRESOL AND OTHER COMPOUNDS TO INSECT EGGS, UNDER LABORATORY AND FIELD CONDITIONS

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(*A joint contribution from the Rothamsted Experimental Station* (*F. T. and C. T. G.*) *and the East Malling Research Station* (*A. M. M.*).)

(With Plate XIII, and 2 Text-figures.)

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In a recent paper (1) the results are recorded of a quantitative examination under laboratory conditions of the toxicity of a number of aromatic nitro compounds to adults of *Aphis rumicis* L. and to the eggs of the Geometrid moth, *Selenia tetralunaria* Hufn. The experiments, which constituted part of a survey of the insecticidal properties of many groups of organic compounds, were made under strictly comparable conditions by means of the spraying apparatus previously described (2). These substances vary widely in toxicity, some possessing insecticidal properties of a very high order and appearing to warrant more detailed investigation.

[1] The names are in alphabetical order.

The *dinitro* compounds and their salts show a higher toxicity than either the mono- or the trinitro-derivatives. Among these, 3 : 5-dinitro-o-cresol was found more toxic than 2 : 4-dinitro-phenol or 2 : 6-dinitro-p-cresol, and was indeed the most active member of the whole series; it is also more readily worked up into a good emulsion suitable for spraying than the other dinitro derivatives tested. For these reasons, special attention has been given to dinitro-o-cresol[1] and its salts.

Laboratory Experiments.

The previous experiments (1) with 3 : 5-dinitro-o-cresol and its sodium salt on the eggs of the Purple Thorn Moth, *Selenia tetralunaria* Hüfn., were repeated, but they were planned so as to yield data suitable for statistical treatment. The moths were reared in large numbers and eggs were obtained laid fairly thickly on muslin. The method of procedure differed from that described in an earlier paper (*loc. cit.*) only in using larger numbers of eggs (50–100) for each test and in triplicating the sprayings at each concentration. As the eggs were unevenly distributed on the muslin, care was taken that, in each set of three sprayings, one piece of muslin was used on which the eggs were very close together, one on which they were less close together, and one on which they were rather widely scattered. In order to obtain as accurate a figure as possible for the percentage of eggs which did not hatch irrespective of treatment, many more eggs were taken for the controls. After spraying and allowing to dry, each piece of muslin bearing the eggs was placed in a separate tube, and when full time had been allowed for hatching, the numbers of hatched and unhatched eggs were counted.

In order to make sure that the benzene used for dissolving the dinitro-o-cresol had no toxic effect upon the eggs, control sprayings with this solvent were included. Concentrations of benzene ranging from 5 c.c. to 0·05 c.c. per cent. were emulsified with a solution of 1 per cent. saponin solution in water. The sprayings were done in duplicate at each concentration and altogether 2214 eggs were used. In addition, five batches of eggs (502 in all) were sprayed with a solution of 1 per cent. saponin and eight batches (654 eggs) were left unsprayed. The average number of eggs which failed to hatch in the benzene-saponin series was 6·41 per cent. (any variations in numbers hatching being quite independent of the concentration of benzene), in the saponin series 6·97 per cent., and in the unsprayed controls 6·88 per cent. Thus there

[1] Throughout the experiments the dinitro-o-cresol used was 1-methyl-2-oxy-3 : 5-dinitrobenzene; and is generally referred to here as dinitro-cresol.

was no evidence of any toxicity due to spraying with the benzene-saponin mixtures or with saponin solution alone. It was therefore considered fair to take all these series together and to regard the mean as the average natural mortality, to be used as a control figure for judging the toxic effects of the compounds tested. This figure was 6·59 per cent. with a probable error of ± 0·23, or 3·49 per cent. of the mean.

In addition to 3 : 5-dinitro-o-cresol and the sodium salt, a commercial "coal-tar distillate," known to have egg-killing properties, was included for comparison, but only one batch of eggs was allocated for each concentration tested. The results are expressed in Table I and Diagrams 1 and 2.

Table I.

Toxicity of 3 : 5-*dinitro-o-cresol and its sodium salt to eggs of* S. tetralunaria.

Substance	Concentration in gm. per 100 c.c.	No. of eggs sprayed	No. of eggs not hatching	Percentage of eggs not hatching	Percentage of viable eggs killed (allowing for control)
Controls					
Unsprayed	—	654	45	6·88	—
Saponin solution	1 % saponin	502	35	6·97	—
Benzene-saponin mixture	5–0·05 c.c. % B. 1 % saponin	2214	142	6·41	—
Mean control figure	—	3370	222	6·59 ± 0·23	—
3 : 5-dinitro-o-cresol	0·2	326	326	100·0	100·0
	0·15	263	263	100·0	100·0
	0·10	272	269	98·9	98·8
	0·08	315	306	97·1	96·8
	0·06	296	272	91·9	91·3
	0·04	280	259	92·5	91·9
	0·02	323	230	71·2	69·1
	0·01	297	93	31·3	26·4
Sodium salt of dinitro-o-cresol	0·35 D.N.C.	254	254	100·0	100·0
	0·2	240	237	98·7	98·6
	0·1	230	222	96·5	96·2
	0·08	296	279	94·3	93·9
	0·06	225	190	84·5	83·4
	0·04	249	214	86·0	84·7
	0·02	207	128	61·9	59·2
	0·01	231	80	34·7	30·1
A tar distillate spray-fluid	10·0	103	103	100·0	100·0
	7·5	97	97	100·0	100·0
	5·0	93	88	94·7	94·3
	3·5	96	91	94·8	94·4
	2·0	61	27	44·3	40·3
	1·0	124	10	8·1	1·5
	0·5	134	6	4·5	0·0
	0·25	84	5	5·9	0·0

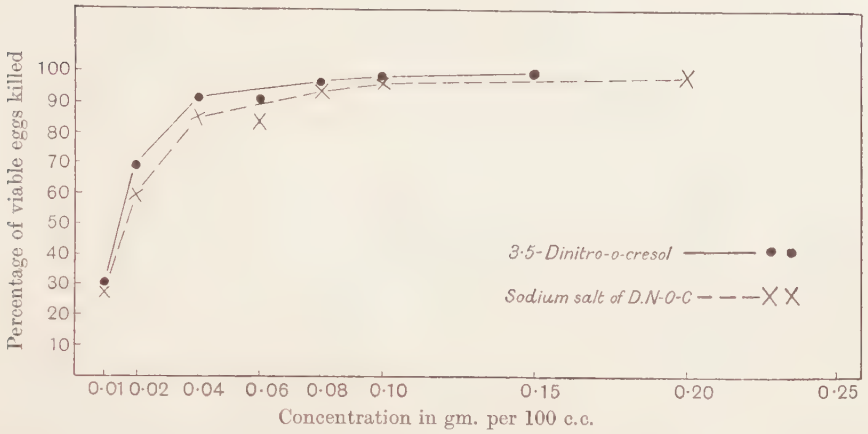


Diagram 1. Toxicity of 3 : 5-dinitro-o-cresol and its sodium salt to eggs of *S. tetralunaria.*

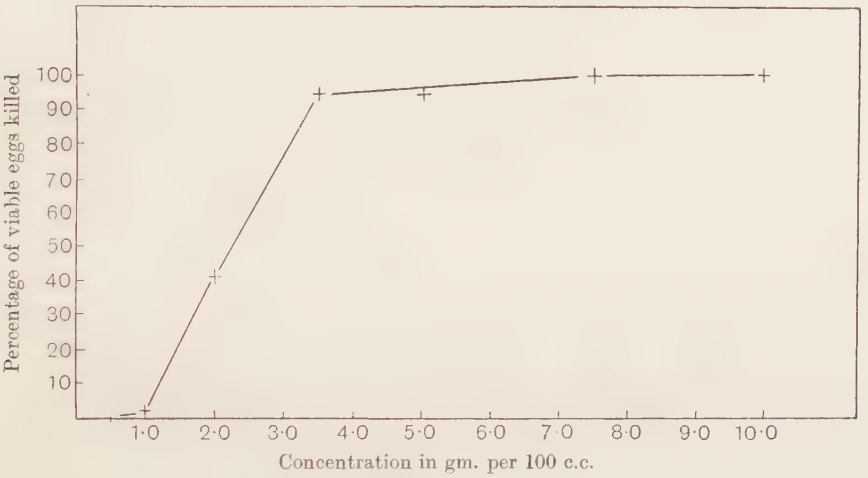


Diagram 2. Toxicity of a tar distillate spray-fluid to eggs of *S. tetralunaria.*

An inspection of Table I and Diagrams 1 and 2 (in which the percentage of viable eggs killed is plotted against the concentration) shows that the high toxicity of 3 : 5-dinitro-o-cresol and its sodium salt to the eggs of *S. tetralunaria* is fully confirmed.

The figures in Diagram 1 are plotted on a larger scale than those in Diagram 2; and this should be noted in comparing the two diagrams. The larger scale of Diagram 1 was rendered necessary by the high toxicity of dinitro-cresol and its salt, and it brings out the slight differences between them.

Mr R. A. Fisher kindly examined the data, and computed that the concentrations at which 50 per cent. of viable eggs were killed were as follows:

3 : 5-dinitro-o-cresol	...	...	...	0·0147 %
Sodium dinitro-o-cresylate	...	...		0·0167 ,,
"Coal-tar distillate" spray-fluid			...	2·3 ,,

The difference in toxicity between dinitro-o-cresol and its sodium salt although probably significant is very small.

Statistically, the concentration which results in 50 per cent. of viable eggs killed is the best for purposes of comparison, and at this point both dinitro-cresol and its sodium salt are much more toxic than the "coal-tar distillate." A comparison of the diagrams shows that the curve for the distillate rises relatively more steeply and therefore at the concentration where 90–100 per cent. of the eggs were killed, the difference in toxicity between dinitro-cresol and the tar distillate is slightly less but still of a very high order.

Additional support is given by these figures to the suggestion made in an earlier paper (*loc. cit.*) that dinitro-o-cresol and sodium dinitro-o-cresylate would be worth trying on a larger scale as egg-killing washes[1]. Although also toxic to adult insects, the use of these compounds must obviously be confined to their application as winter spray-fluids on dormant trees and bushes since they cause serious damage to foliage, even at very low concentrations. There is no prospect of compounds of this type being used for summer spraying; attention has therefore been concentrated on their egg-killing properties.

Before however giving an account of some experiments carried out on a field scale, it is necessary to refer to the effect of dinitro-cresol upon the eggs of some insects other than those of *Selenia tetralunaria* already mentioned. A small-scale experiment with the eggs of the Winter Moth (*Cheimatobia brumata* L.), which are known to be highly resistant, showed that 5·4 per cent. only (12 out of 233) gave rise to living larvae after treatment with dinitro-cresol at a concentration of 0·25 per cent.; whereas untreated controls from the same lot of eggs gave a hatch of 98 per cent. (284 out of 290).

[1] As stated in the paper already referred to, the potassium salt of dinitro-o-cresol was put on the market as an insecticide in the form of a paste mixed with soap, some 30 years ago, and was recommended as a general spray for the control of biting and sucking insects. This was not known to us at the time of the original experiments and as far as we are aware, very little has been heard of this material for many years; it is probable that it fell into disrepute on account of its injurious action on foliage of all kinds.

Other eggs available for laboratory scale tests were those of two species of Aphides, the Glasshouse Tomato Moth (*Hadena oleracea* L.), the Magpie Moth (*Abraxas grossulariata* L.), the Vapourer Moth (*Orygia antiqua* L.) and *Tortrix pronubana* Hb.; dinitro-cresol was highly toxic to all these species. Evidence has also been obtained that washing eggs of *S. tetralunaria* in a rapid stream of water a few hours after treatment with a toxic concentration of dinitro-cresol does not reduce the effect.

These experiments are of a preliminary nature and further work is in hand, but they suffice to show that dinitro-cresol is not narrowly specific in its action.

Field Experiments.

Considered as a whole, the results of the laboratory experiments so far discussed appeared to justify an attempt to ascertain whether 3 : 5-dinitro-o-cresol and its sodium salt would prove effective under practical conditions as an egg-killing winter spray fluid. A series of field scale trials was therefore planned, to be carried out at the East Malling Research Station; and the opportunity was taken to test a method of measuring accurately the effect of such spray fluids in the field which was under consideration at that Station.

Introduction.

Since the effects upon living plants of some of these possibly injurious compounds were not known, it was necessary to select trees for the experiments that were not required for further work.

Fortunately there was available on one of the pathological plots a number of four-year-old plum root stocks of known varieties; a few of each of these had been grafted with the variety Victoria. They had been allowed to grow naturally, and owing to their close proximity to one another, and to the thickness of their growth, they were not particularly easy to spray efficiently. One further disadvantage of this material was that it consisted of nine varieties planted in single rows so that it was not practicable to treat each variety with each spray fluid. However, as will be pointed out later, this was not a very important matter, since all the varieties of plum appeared to be equally heavily infested with aphis and to be equally resistant to spray damage at the period of application. Moreover, it was possible with one exception to arrange the plots in such a manner that the majority included at least one or two of the Victoria trees already mentioned.

On the other hand, this material had certain very marked advantages. First, in other experiments with tar distillate washes, plums have been found to show susceptibility to damage, especially from unduly late spraying. If any damage had resulted, these trees might have been expected to show whether there were varietal differences in susceptibility.

In the second place, the trees were infested with large numbers of the eggs of the Hop-Damson Aphis (*Phorodon humuli* Schr.) and Red Spider (*Tetranychus telarius* L.), with a sprinkling of other eggs, in a season when, in the immediate vicinity of East Malling, insect infestations were notably scarce.

Examination both of individual shoots, and of each of these trees as a whole, indicated that the infestation of Hop-Damson Aphis was very general. The distribution of infestation is illustrated in the table below.

Table II.

Illustrating distribution of infestation of Hop-Damson Aphis (Phorodon humuli *Schr.*).

Variety	Number of shoots examined	Total number of aphis eggs counted	Average per shoot
Common Mussel	10	1173	117
Myrobolan B.	10	1346	135
Pershore	10	1392	139
Victoria	10	1192	119

Since the material available contained at least three species of *Prunus*, this general infestation is rather surprising, but it was probably accounted for by the proximity of the plots to a hop garden, from which the aphides had migrated during early autumn.

The detailed observations were mainly confined to the effects of the spray fluids upon eggs of the Hop-Damson Aphis.

Field methods of judging results.

As already mentioned, a point of special interest in these experiments was the elaboration, under field conditions, of an accurate method for determining the comparative effects of these and similar washes.

The method commonly used in the past has been to judge by general impression the amount of insect damage to each tree in the season following the application of the spray. This method of judging results had been tried by one of us during the previous season in preliminary spraying trials with winter washes and had been found unsatisfactory.

Although "general impression" was valuable in determining the cleansing effect upon the tree, lack of spray injury, etc., it did not afford any measurable data as to actual egg-killing effects. Nevertheless, the experience of the past season had taught that, if a survey of the actual egg population was to be undertaken, it should be on a sufficiently large scale to allow for the probable interference of birds, predaceous insects, etc. A system was therefore worked out whereby quantitative surveys of the insect eggs could be made before spraying, and during the hatching season.

When the detailed examinations of the plum shoots began, a practical difficulty at once arose owing to the immense numbers of Hop-Damson Aphis eggs, which made a complete survey impossible. Consequently, on each tree, shoots from twelve to eighteen inches in length were examined, and a single one selected which showed a "countable" number of eggs. This shoot was marked, and as far as possible a survey was made of the eggs on it. Since even on these selected shoots it was generally impossible to count all the eggs, it was necessary to take only those in recognisable places, *e.g.* in close proximity to the buds, on petiole scars, in cracks and wounds on the internodes, and on short spurs. On very dull days and when the shoots were wet it was impossible to count the eggs.

After spraying, daily examinations for hatchings were made both on sprayed and unsprayed trees. In order to facilitate this work, shoots adjacent to the selected ones were cut off the trees and examined in the laboratory from time to time. These examinations showed that when approximately 55 per cent. of the aphis eggs on the unsprayed trees had hatched, the rest (with few exceptions) had already shrivelled. It was judged that this was the critical moment for a post-spray examination of the marked shoots, since if all the eggs capable of hatching on the control trees had hatched, those on the sprayed trees should have done the same.

All shoots on which eggs had been previously counted were now cut off and examined in the laboratory, the shoots from the control trees being examined first in order to give the eggs on the sprayed trees every opportunity to hatch.

Further, in order to give the eggs from the trees sprayed with different fluids equal time to hatch, twigs from each of the plots were examined on one day, and the process repeated until all of the eggs had been counted. Although this precaution was taken, it was noted at the time that the percentages of eggs hatched were not materially higher

Table III.

Illustrating method of recording aphis eggs on individual shoots, and close correspondence of the two surveys.

(*Page from field record book.*)

	Pre-spray count in plantation		Final post-spray examination in laboratory	
			Hatched	Shrivelled
Basal bud	1		0	1
2nd ,,	(2)		(1)	(1)
3rd ,,	7		3	4
4th ,,	5		2	3
5th ,,	(2)		(1)	(1)
1st Lateral shoot				
Basal bud	5		0	5
2nd ,,	1		0	1
3rd ,,	[4]		[1]	[3]
4th ,,	1		0	1
5th ,,	3		0	3
2nd Lateral shoot				
Basal bud	2		2	0
2nd ,,	0		0	0
3rd ,,	1		0	1
4th ,,	1		1	0
3rd Lateral shoot				
Basal bud	6		3	3
2nd ,,	3		1	2
3rd ,,	[2]		[0]	[1]
4th ,,	4		1	1
5th ,,	4		1	1
6th ,,	7		0	7
7th ,,	1		0	1
6th bud	10		4	6
4th Lateral shoot				
Basal bud	4		1	3
2nd ,,	0		0	0
3rd ,,	0		0	0
4th ,,	0		0	0
5th ,,	2		0	2
6th ,,	1		0	1
7th ,,	1		1	0
8th ,,	0		0	0
5th Lateral shoot				
Basal bud	10		5	5
2nd ,,	0		0	0
3rd ,,	4		0	4
4th ,,	0		0	0
5th ,,	0		0	0
6th ,,	0		0	0
7th bud	8		3	5
8th ,,	12		2	10
9th ,,	12		3	9
10th ,,	6		0	6
11th ,,	9		4	5
12th ,,	(4)		(1)	(3)
13th ,,	6		2	4
14th ,,	8		4	4
15th ,,	5		0	5
Total originally noted	164	Total ultimately accounted for	47	112

on shoots examined later. Hence it may be stated confidently that the normal hatching period was complete before the final examinations were undertaken[1].

During the second survey of the shoots, the chief points looked for were, first, the number of empty egg cases from which aphides had hatched; and second, the unhatched or shrivelled eggs. As will be seen from Figs. 1 and 2 (see Plate XIII) there was little difficulty in distinguishing between the hatched and shrivelled eggs, the final count being made under a binocular microscope. A pocket lens had to be relied upon for the pre-spray survey (made in the field). A typical page from the record book (Table III) shows how closely the pre-spray and post-spray surveys corresponded with one another.

The success or failure of this method of procedure must depend upon the possibility of re-locating the eggs with certainty. It would be no easy matter to find small groups of eggs a second time unless they could be retraced by "landmarks" such as buds, petiole scars and cracks in the internodes. The counts of eggs always commenced at the bud nearest the base of the shoots, and each bud was taken in order up to the apex. If the eggs around any one bud occurred in very large batches, they were not counted, but a reference was made in the field book noting their presence in quantity. In order to make identification easy, all eggs recorded on petiole scars were indicated (as shown in Table III) by ordinary brackets, while those found in cracks or wounds were distinguished by square brackets.

Even by following these methods, it was not anticipated that 100 per cent. of the eggs recorded at the first survey would be accounted for, since it was probable that casualties from unknown causes would take place. In the particular instance given in Table III, approximately 3 per cent. of the eggs could not be found at the second count. Over the whole survey, the percentage of loss from unknown causes was 6 per cent. This percentage is likely to vary from season to season and from experiment to experiment, and since the mortality from natural causes, as well as the percentage of "unhatchable" eggs must be allowed for, the pre-spray count of eggs would appear to be amply justified. In the series of experiments under discussion this loss of eggs from unknown causes was not large enough to interfere seriously with the comparison of the proportions hatched on trees under the various treatments.

In addition to the detailed records on the selected shoots, general observations were recorded on each tree. In the first of these surveys,

[1] The final counts were not made on the sprayed shoots until three weeks after all hatchings had ceased on the control shoots.

quantity and species of eggs on the stems and stout shoots were noted, not individually, but generally. These general notes proved valuable later, since they confirmed the impression that the infestation of the Hop-Damson Aphis was widespread over the plots. After selected shoots had been fully examined, a second general survey of each plot, made after spraying, showed Hop-Damson Aphis eggs to be hatching freely all over certain plots, whereas on others such was not the case. By this means it was possible to confirm generally the effect of the sprays already measured on individual shoots. A third general survey revealed the fact that certain eggs, for example those of Red Spider, were not affected by the spray fluids.

It was also possible at the same time to get some idea of the cleansing properties of the washes, and some measure as to the amount of damage to leaves and shoots caused by Hop-Damson Aphides which hatched.

Natural mortality in the field.

In order to measure the effects of the spray fluids accurately, it was essential first to arrive at a figure for the normal mortality of eggs on unsprayed trees so as to have adequate controls. For this purpose,

Table IV.

Natural Mortality in the Field.

Shoot No.	Percentage of recorded eggs not hatching	Shoot No.	Percentage of recorded eggs not hatching
1	49	27	49
2	45	28	47
3	31	29	45
4	46	30	48
5	48	31	43·5
6	17	32	42
7	45	33	44
8	46	34	44
9	46	35	44
10	46	36	48
11	48	37	47
12	48	38	43
13	49	39	48
14	46	40	47
15	44	41	42·5
16	44·5	42	44
17	48	43	46
18	40·5	44	47
19	47	45	46
20	46	46	44
21	47	47	45
22	49	48	45
23	46	49	48
24	50	50	46
25	48	51	47
26	44		Average 45

51 convenient shoots (from 41 unsprayed trees) were selected, and in all 9446 eggs were counted and recorded.

Table IV shows the actual percentage of eggs which failed to hatch on each shoot.

It will be seen that these percentages are remarkably uniform. The figures for shoots Nos. 3 and 6 are exceptional, but the discrepancy is less important than it appears since these two shoots had only 45 and 82 eggs respectively whereas the number of eggs per shoot for the whole series averaged 185.

This uniformity is further confirmed by the probable error of the mean. This works out at $\pm$ ·47 or slightly over 1 per cent. of the mean.

Arrangement of plots.

Details with regard to the trees used for the experiments have already been given. Of the 137 trees available, 41 were reserved as "controls." The majority of these were distributed in four large plots and the remainder isolated.

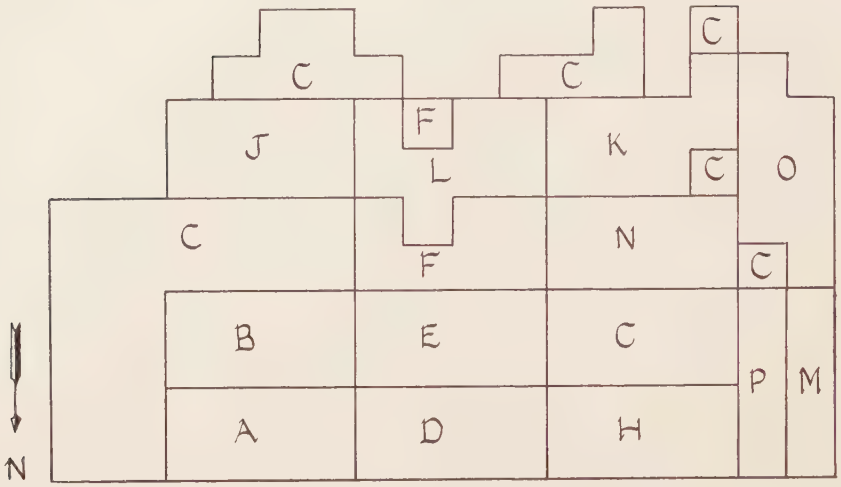
With two exceptions, the sprayed plots each consisted of eight adjacent trees, arranged, as far as possible, to comprise two varieties of plum stock, and, in addition, at least one Victoria tree. In three treatments only (namely plots B, K and P) was it impossible to include a Victoria tree, and on a single plot (P) it was only possible to include one variety of root-stock (Pershore). Since it was desirable to test as many sprays as possible, two of the less important fluids were allotted only four trees each (plots M and P).

A map is given (p. 458) showing the distribution of these plots on the land.

Preparation of spray fluids.

The field trials were limited mainly to testing 3 : 5-dinitro-o-cresol and sodium dinitro-o-cresylate; but for purposes of comparison, single plots treated with chlordinitrobenzene, an emulsified "heavy oil," and a proprietary tar distillate spray were included.

3 : 5-dinitro-o-cresol and chlordinitrobenzene are only slightly soluble in water, and some organic solvent is necessary for the preparation of an emulsion suitable for spraying. In the laboratory experiments benzene was suitable; but it was obvious that this solvent could not be used in the field, on account of its inflammability and volatility. Other solvents were therefore sought and it was finally decided to use (1) one of the lighter fractions of the products of tar distillation known com-



Arrangement of Plots.

The treatment of each plot can be ascertained by reference to the following key:

Plot	Treatment: Substance		Treatment: Solvent	
A	Dinitro-o-cresol... ...	0·25 %	High flash tar oil ...	2·5 %
B	„ „	0·5	„ „ ...	5·0
C	Unsprayed Control			
D	Dinitro-o-cresol... ...	0·35	Emulsified disinfectant	3·5
E	„ „	0·25	„ „	2·5
F	„ „	0·15	„ „	1·5
H	Sod. dinitro-o-cresylate	0·35*	Water (with 0·5 % soap)	
J	None		Emulsified disinfectant	3·5
K	None		„ „	2·5
L	None		„ „	1·5
M	Chlordinitrobenzene ...	2·5	High flash tar oil ...	25·0
N	Emulsified heavy oil ...	7·5	None	
O	Proprietary tar distillate	7·5	None	
P	None		High flash tar oil ...	5·0

* Calculated as dinitro-cresol.

mercially as "high flash tar oil" and (2) an emulsified tar distillate product sold as a disinfectant, which for purposes of reference we have termed "an emulsified disinfectant."

Stock solutions were prepared of a fairly high strength, the appropriate dilutions being made on the field immediately before use. Dinitro-o-cresol was readily soluble in both the high flash tar oil and the "emulsified disinfectant," stock solutions of 10 per cent. strength being

rapidly prepared by stirring the moist crystals with the requisite amount of solvent. The following important difference, however, attended their use. In order to obtain a fairly stable emulsion which would spread and wet well, a half per cent. solution of soap in water was used for dilution of the stock solution in high flash tar oil; double decomposition of the soap followed giving rise to some sodium dinitro-cresylate and fatty acids. On the other hand, the stock solution in the "emulsified disinfectant" could be diluted directly with water to give a stable emulsion of unchanged dinitro-o-cresol, eminently suitable for spraying.

On plots A and B therefore, where the solvent used was high flash tar oil, the spray fluid as finally diluted contained an unknown amount of sodium dinitro-cresylate and fatty acid. The total concentration, calculated as dinitro-cresol, was not however thereby altered.

The stock solution of sodium dinitro-o-cresylate was prepared as a solution in water, at a strength equivalent to 4·5 per cent. dinitro-o-cresol, by stirring the dinitro-o-cresol with a solution of carbonate of soda, at a strength slightly in excess of that required for neutralisation. The liquid for spraying was obtained by dilution of the stock solution with 0·5 per cent. of soft soap in water. Of the other chemical materials used, chlordinitrobenzene was dissolved in high flash tar oil and the stock solution (10 per cent.) diluted with soap solution, no double decomposition being observed. Emulsified heavy oil, a by-product of the Mond gas process, was diluted with water only to give a spray-fluid suitable for use. The "emulsified tar distillate spray fluid," a commercial product largely used in recent years as a winter-wash, also required dilution with water only.

Methods and conditions of spraying.

The growing conditions of the trees already described necessitated the use of spraying machinery which could easily be handled amongst crowded trees. A hand manual spraying machine was therefore used. The spraying pressure obtained was from 75 to 80 lbs. to the square inch; since none of the trees was above 10 feet in height this proved quite effective.

In order to guard against any of the sprays drifting from one plot to another, a screen of thick hessian cloth, nearly twice the height of the trees, was placed round three sides of each tree during spraying, leaving the operator just room to work. This successfully prevented any serious drifting of the sprays.

All sprays were applied on December 22nd, 1924.

The weather conditions were admirable. Although it was cloudy in the morning, the sun subsequently appeared in the afternoon, and shone for three hours. The following day, however, the conditions were not favourable; after two hours' sun in the morning, half an inch of rain fell during the late afternoon and evening. Rain again fell on the following four days, and as much as 0·63 of an inch was recorded on the last of them. It will be seen, therefore, that although the conditions were favourable on the actual date of spraying, the adverse weather which followed might have been expected to be detrimental to the effect of the sprays.

Very mild weather conditions prevailed during January, and in consequence some eggs began to hatch at an early date. The first record of hatching on cut shoots in the laboratory was on January 9th. In the field, the first eggs hatched on January 19th. The eggs hatched mainly from "control" shoots, but observations showed that two of the sprayed plots (K and N) also showed hatching at this early date.

Discussion of results.

The final figures obtained by the methods described are summarised in Table V.

Table V.

Field experiments with eggs of Hop-Damson Aphis.

Plot	Treatment: Substance	Treatment: Solvent	Total No. of eggs examined	No. hatched	No. not hatched	Per cent. not hatched	Per cent.* killed allowing for control
C	Controls		9446	5171	4275	45	—
D	Dinitro-o-cresol ... 0·35 %	Emulsified disinfectant† 3·5 %	912	104	808	89	80
E	„ „ ... 0·25	„ „ 2·5	770	84	686	89	80
F	„ „ ... 0·15	„ „ 1·5	1049	117	932	89	80
J	None	„ „ 3·5	918	453	465	51	11
K	None	„ „ 2·5	975	651	324	33	Nil
L	None	„ „ 1·5	1386	812	574	42	Nil
B	Dinitro-o-cresol ... 0·5	High flash tar oil ... 5·0	1147	110	1037	90	82
A	„ „ ... 0·25	„ „ ... 2·5	1423	136	1287	90	82
P	None	„ „ ... 5·0	742	252	490	66	38
H	Sodium salt of dinitro-o-cresol 0·35 % (calcd. as D.N.C.) ...	Water with 0·5 % soap	1246	1114	132	89	80
M	Chlordinitrobenzene 2·5 %	High flash tar oil ... 25 %	299	73	226	76	56
N	Emulsified heavy oil 7·5	None	974	533	441	45	Nil
O	Proprietary tar distillate 7·5	None	1131	151	980	87	77

* The control figure has been allowed for in each case by deducting the average percentage which did not hatch on the control plots (45 %) from the percentage not hatched on each sprayed plot and multiplying by $\frac{100}{100-45}$.

† This product was a proprietary article sold as a disinfectant.

A reliably accurate control was obtained by examining a large number of eggs (9446) on the untreated plots. The average figure for the mortality from natural causes was 45 per cent. (cf. also Table IV) and only the remaining 55 per cent. can be considered in comparing the effects of the treatments. The figures given in the last column of Table V represent the percentage of "hatchable" (viable) eggs killed, allowance being made for the natural mortality; they thus give a quantitative measure of the comparative efficiencies of the treatments under the conditions of the experiment.

The high toxicity of 3 : 5-dinitro-o-cresol, found in the laboratory experiments already referred to, is confirmed under field conditions. Five plots (D, E, F, B and A) were sprayed with fluids containing this compound at concentrations ranging from 0·5 per cent. to 0·15 per cent. and in every case 80–82 per cent. of the viable eggs were killed. The two solvents for dinitro-o-cresol used in making up the spray fluids were tested alone on plots J, K, L and P, and found to have no effect at the lower strengths used and but little effect at the highest strength.

Sodium dinitro-o-cresylate, at a concentration of 0·35 per cent. (calculated as dinitro-cresol) dissolved in water containing 0·5 per cent. of soft soap (plot H) also killed 80 per cent. of the viable eggs. This result agrees with the laboratory experiments on eggs of *Selenia tetralunaria* already discussed, which showed only a very slight difference in toxicity between dinitro-cresol and the sodium salt. From the practical point of view, the salt appears to have certain advantages, as it is soluble in water to the extent of about 5 per cent. and permits of use in soap solution since it does not liberate free fatty acid. On the other hand, preliminary laboratory experiments indicate that, after application and allowing to dry, it is more readily removed by washing with water, and might not therefore be so effective under wet weather conditions.

A proprietary tar distillate spray fluid, used at the strength recommended (7·5 per cent.) was included for purposes of comparison (plot O), and at this strength 77 per cent. of the viable eggs were killed, a result of the same order as that given by dinitro-cresol. Since none of the treatments which proved effective gave a higher figure than about 80 per cent. viable eggs killed, and since, in the case of dinitro-cresol, this figure was obtained both at the highest and lowest concentrations used, it seems probable that this value represents a kill of 100 per cent. of the eggs which actually came in contact with the fluid. The spraying was done with all possible care but under field conditions some crevices and sheltered places, in which eggs were laid, will always be liable to

escape wetting. If these assumptions are correct, it follows that a kill of about 80 per cent. of eggs would be the maximum obtainable under ordinary field conditions. This deduction seems unlikely, and is rendered improbable by the condition of the trees in the following spring upon plots where this figure was obtained. As compared with the control plots, they showed a freedom from aphides, which would not have been expected had 20 per cent. of the viable eggs survived, suggesting that appreciably more than 80 per cent. of the total number of the viable eggs had failed to hatch. A probable explanation is provided by some observations made in laboratory experiments with eggs of several species of moths. With substances as toxic as dinitro-cresol, it not infrequently happened that with very low concentrations, or in cases where the eggs were not thoroughly wetted, hatching was not prevented, but the young larvae were killed immediately on emergence, due to contact with or absorption of traces of the poison. It is reasonable to assume that this also occurred in the case of a proportion of the aphis eggs in the field experiments. In our counts such eggs would necessarily be included in the number recorded as "hatched," whereas *in effect* they belong to the "not hatched." The final figure for the percentage killed, at least on the dinitro-cresol plots, is thus probably below the truth.

It is further possible that, in so far as the condition of the trees during the spring was affected by re-infestation from neighbouring trees (on control plots, etc.), migrating aphides may have been deterred from settling where traces of the spray fluid remained.

Of the other materials included in the trials, chlordinitrobenzene at the comparatively high concentration of 2·5 per cent. (plot M) killed only 56 per cent. of the viable eggs and does not seem worth further consideration from a practical point of view. The emulsified "heavy oil" at 7·5 per cent. (plot N) was quite ineffective.

The figures given in Table V considered as a whole, show that the quantitative method adopted has given a reliable comparison between the efficiency of the various treatments. The results are consistent—compare, for instance, plots D, E and F (dinitro-cresol and solvent) with plots J, K and L (solvent alone); and the extreme differences in the percentage of viable eggs are very large.

Calculation of the probable error indicates that at all events the bigger differences are unquestionably significant. The percentage difference between the number of eggs hatched on control plots and on plot A amounts to $43{\cdot}8 \pm 1{\cdot}23$, *i.e.* the difference is more than 35 times its probable error. In this type of experiment, a difference about

three times its probable error is frequently considered significant. The large differences recorded in this series of experiments can therefore be accepted without hesitation, at least for the particular seasonal conditions under which they were carried out.

With regard to the effects of the sprays upon other insect eggs the following brief records were made. Red Spider eggs were numerous on all plots. In the spring, frequent examinations showed that the mites readily hatched out from their eggs on all plots, and were commonly seen on the trees throughout the summer. It would appear that none of the spray fluids, at the concentrations used in this investigation, had a toxic effect on Red Spider eggs. One of the plum gall mites (*Eriophyes phloeocoptes* Nal.), which hibernates under the bud scales, could be found in reasonable numbers only on the control plots. Hence it appears that the mites were probably killed by the sprays.

General observations.

All the treatments showing a high efficiency against the eggs of the Hop-Damson Aphis (plots A, B, D, E, F, H and O) also had a marked general cleansing effect upon the trees. All lichens and mosses on the trunks were killed and the bark thoroughly cleaned, giving the tree a healthy appearance. The "emulsified disinfectant" solvent alone at the two higher strengths (J and K) had a similar cleansing effect. The lowest strength of this solvent used (plot L) and the other solvent (high flash tar oil, plot P) had no cleansing action, the trees on these plots being scarcely distinguishable in appearance from those on the unsprayed (control) plots.

During the following spring and summer, field notes were made on the spread of the Hop-Damson Aphis on the trees. "Control" trees could be readily picked out; they were more severely damaged by aphis than those on the sprayed plots. Curled leaves were prevalent on all these trees, and the new growths were much stunted. Plot K ("emulsified disinfectant," 2·5 per cent.) was markedly affected by aphis attack; curled leaves were numerous, and many of the new growths were stunted. Plots L and N (emulsified disinfectant, 1·5 per cent.; and emulsified heavy oil, 7·5 per cent.) were also severely attacked by aphis, but less than plot K. Aphides were commonly found throughout the spring on plots M and P (chlordinitrobenzene, 2·5 per cent.; and high flash tar oil, 5·0 per cent.). On these trees the infestation was not evenly distributed, some shoots being more severely attacked than others; nevertheless none of the trees were free from aphis.

Of the remaining plots, those treated with dinitro-cresol and

sodium dinitro-cresylate were, with few exceptions, free from aphis throughout the summer. The slight infestation on these plots can probably be accounted for by migration of aphides from adjoining plots; it was possible to find small colonies established on some of the trees on plots generally free from them.

Finally, a survey of the plots was made both before and after blossoming, to ascertain whether the trees themselves suffered as a result of the application of the sprays. These examinations showed that, with the exception of one tree, there was no apparent spray injury, on any of the plots, a satisfactory result seeing that plums are well known to be very susceptible to spray damage. It is reasonable to conclude that any spray likely to cause "spray injury" under dormant conditions would have done so in these experiments. One tree on plot M (chlor-dinitrobenzene) appeared to be very unhealthy during the latter part of May. The leaves turned yellow, and the tree was defoliated early in the season. Whether this tree was injured as a result of the spray is uncertain. The remainder of the trees on this plot grew normally throughout the season.

The writers' acknowledgments and thanks are due to Mr R. H. Le Pelley, B.Sc. for his assistance in carrying out the field experiments, particularly in connection with the laborious task of counting and recording the aphis eggs.

Conclusions.

The method adopted[1] for obtaining quantitative data as to the egg-killing powers of different substances under field conditions has proved satisfactory. It was possible, with the material available, to compare the efficiency of the various treatments in the field with an accuracy comparable with that of the laboratory experiments; and the figures obtained under the two sets of conditions are in general agreement.

The results of the laboratory and field experiments taken together show the high insecticidal value of dinitro-o-cresol and its sodium salt and make it very desirable to carry out further work from the point of view of the use of these compounds in practice as winter spray-fluids. The trials have given very positive evidence of their efficiency in the field against the eggs of the Hop-Damson Aphis; and it is hoped to obtain experimental confirmation of these results under differing conditions.

A number of points need further investigation. For example, more information is wanted as to the effect of these two substances in the

[1] A paper by F. Z. Hartzell has recently appeared on "Susceptibility to dust and spray mixtures of the Pear Psylla" (New York Agric. Exp. Sta., *Bull.* 527, July 1925), in which a similar method is described.

Fig. 2.

Fig. 1.

GIMINGHAM, MASSEE & TATTERSFIELD.—A Quantitative Examination of the Toxicity of 3 : 5-dinitro-o-cresol. (446—465.)

field upon the eggs of insects other than aphides, and as to their safety and economy in use under all conditions[1]. Again, the most suitable method for preparing a spray-fluid with 3 : 5 dinitro-o-cresol as the active constituent has yet to be finally determined.

Summary.

1. The toxicity of 3 : 5-dinitro-o-cresol and its sodium salt to eggs of the moth *Selenia tetralunaria* Hüfn. has been determined quantitatively under controlled conditions in the laboratory. The figures obtained confirm earlier results and show (*a*) that these compounds have a very high toxicity to insect eggs, and (*b*) that the sodium salt of dinitro-o-cresol is only slightly less toxic than dinitro-o-cresol in the uncombined state. The relationship between concentration and toxicity is expressed by means of diagrams.

2. Preliminary laboratory experiments indicate that dinitro-o-cresol is also highly toxic to insect eggs of a more resistant type than those of *S. tetralunaria*.

3. Spray fluids containing dinitro-o-cresol or the sodium salt showed a high efficiency against eggs of the Hop-Damson Aphis (*Phorodon humuli* Schr.) on plum trees on a larger scale under field conditions. The trees sprayed with these compounds remained almost free from aphides during the following spring when the control trees were badly infested.

4. A quantitative method for judging the results of the field experiments was worked out. This involved recording details of large numbers of eggs on selected shoots on sprayed and control trees before and after spraying, a numerical measure of the effect of the various treatments being thus obtained. The method gave consistent and reliable results.

5. The spray fluids containing dinitro-o-cresol and its sodium salt had a marked general cleansing effect on the trees. No injury to the trees was observed.

[1] (Note added 12th May, 1926.) Since this paper was handed in for publication, a considerable amount of additional data has been obtained, both in the laboratory and in the field, as to the high toxicity of dinitro-o-cresol and its sodium salt to the eggs of a number of other insects and as to their safety in use as winter spray fluids upon various fruit trees. It is hoped to publish details of this work at an early date.

DESCRIPTION OF PLATE XIII.

Fig. 1. Unhatched eggs.
Fig. 2. Shrivelled and hatched eggs.

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(*Received March 1st*, 1926.)

DISCUSSION ON "THE PLACE OF THE SYSTEMATIST IN APPLIED BIOLOGICAL WORK"

Ordinary Meeting of the Association, Friday, November 20th, 1925, Prof. V. H. Blackman, F.R.S., President, in the Chair.

Dr J. Waterston (British Museum: Nat. Hist.), in opening the discussion, said:

It would be an engrossing and I believe a not unprofitable preliminary adventure, in dealing with a subject like the present, to trace in some detail the part played in the past by the systematic worker in the field of biological enquiry and to note the varying estimates delivered on the results of his labours. To give such a *résumé* even in outline cannot be attempted now. Yet the most cursory acquaintance with the history of Biology should convince us of the enormous debt which every branch of the science, morphology, ecology, genetics and biochemistry, to mention only a few, owes to the students who first gave names to plants and animals and sought to put in orderly array the facts known about them. Too often this point is obscured. In certain quarters it is the fashion to decry systematic botany and zoology, either because of their supposed lack of interest or for their imperfect performance or on more fundamental grounds. As regards the first objection it is enough to say surely that the study of systematics is a matter of taste. The thirst for knowledge—that striving for intellectual satisfaction—does not (fortunately) drive all within the same field in the same direction. Strange as it may seem to some, the systematist loves his task, nay further I will say he is an enthusiast in his work; without that quality he would neither take up so formidable a task nor endure its drudgery. "Poeta nascitur non fit." The systematist "nascitur et fit." For if you add experience to his equipment you have the two most essential qualifications of the systematic biologist.

As regards the second charge—touching the quality of systematic work—so far as it is true my fences are down. There has been in the past and there may be in the future an appalling amount of bad systematic work. Irrelevant descriptions, inaccurate and misleading dichotomies, impossible affiliations, grotesque groupings of biological entities, clutter and cumber the ground and reduce not merely the neophyte to despair. But equally there have been comprehensive penetrating illuminating masterly minds at work, considering the data, weighing, assimilating and marshalling them in monographs which have stood the test of time and become the indispensable basis of any subsequent enquiry referring to the organisms dealt with.

But there are more fundamental doubters. Modern systematics dates from the tenth edition of Linné's *Systema*, and the general adoption of binominal nomenclature. Naturalists agreed, *i.e.*, to refer to species by *two* names instead of one.

This was a radical departure and though I cannot pause to comment on the significance of so deceptively simple a step, its results may be noted. In brief, the true method for an orderly accumulation of biological facts had been discovered. Great and immediate advances were effected, and from 1758 the continuous progress of modern biology dates. Following Linné came the palmy days of the older systematists. Animals and plants had to be named and arranged in some sort of order. Classifications in different groups had to be formulated and tested, and this occupied several decades. But as time went on many branches of enquiry budded off from the parent stem. In systematics itself the age of specialisation set quickly in with its gains and losses. Biology became divided sometimes against itself and the children rose up to call their parent anything but blessed. I have heard it seriously argued that the numerous subdivisions that have taken place in biology have made systematic enquiry obsolete, and how can one classify species when one neither knows what constitutes a species nor can define its limits in any particular case. Doubts of this kind are fundamental, yet they admit of a fairly ready answer. No imaginable widening of the biological horizon, no opening up of new fields of enquiry, no new discovery, *e.g.* in the laws of inheritance, can make unnecessary the work of the systematist, though they make it much more valuable. For the older enquiry deals with organisms as *wholes* and not departmentally. The systematist again may not know what absolutely a species is but he has consciously or unconsciously a working hypothesis which he applies in practice, amending his definitions as experience may show to be necessary.

Now on any of the points on which I have touched considerable discussion might arise. They have been mentioned not for that purpose but solely to throw into relief what I take to be the true idea of a modern systematist and his work. He finds in nature communities or groups of similar individual organisms which he calls species and he makes it his business to arrange these and to summarise the salient facts about them in the simplest and most intelligible form. Primarily he is a morphologist, with an eye both for the general and local form of his species and a keen appreciation of comparative differences. While he studies specially external features and characters, those that are internal are not neglected. The scalpel and the microscope are his friends. Should occasion arise he may employ the results of more specialised studies and become debtor now to the Greek murmuring of genes, determinants or mutations in his slumber, now to the barbarian who has discovered some new tropism while spraying his potatoes.

It does not greatly trouble me that so comprehensive an ideal of the systematist's qualifications is not everywhere entertained. It seems obvious that, by the inevitable logic of events, we are progressing to this goal. Yet we must gratefully acknowledge what has been accomplished by far less perfectly equipped workers. Excellent results have often been achieved by the amateur with no more than enthusiasm, dogged determination, and an eye for a species. But more is demanded at the present day. The formative period in systematics is over. New genera and new species it is true continue to arrive in abundance, but very rarely indeed is a plant or animal discovered necessitating any major modifications of existing schemes. The new arrivals automatically fall into line.

I can imagine someone who has come with me so far interrupting at this point. Whether the systematist as you have described him exists or is merely evolving I have a much simpler definition to offer. Here am I with my particular problem revolving

round the habits, activities or metabolic products of this organism. What is it? Can you tell me its name? Any one who can do this for me I shall be glad to acknowledge as an authority. He and no other is the only systematist whose work has any bearing on applied biology. Granted. The point must be taken up later, but notice its implications. It is no isolated phenomenon. Everywhere in my mind's eye is a picture of the systematist sitting solitary in his study or laboratory and at the door a queue of earnest seekers with one thought in their minds and one question in their eyes: "What is it?"

While one would not for a moment discourage such requests or regard them as illegitimately made the time has come to draw attention to the fact that in a great many departments, especially in entomology, the supply of systematists is quite inadequate to meet the demands which the applied branches are making on them. In most cases an immediate answer is wanted and it is the time factor which is decisive. The past quarter of a century has seen a great army of biologists recruited for the service of medical and veterinary research; for investigations under Boards of Fisheries and Forestry; for advisory work in connection with the growing of the raw material of industry, and there has been no parallel or commensurate development on the systematic side. The most pressing need to-day in biology is for more and better systematists. To attempt to supply the need from amateur ranks is neither possible nor desirable. If the work of the systematist is to be as exacting as I have pictured to you, we must have it taken up by graduates of some standing, and our universities will have to move in the matter and provide ampler facilities for training. To take one instance, there is nowhere in this country a chair of systematic entomology, where one might learn something of the principles of the art of classifying insects, and the result is that a student whose life work may be the arranging or control of insects comes down so ill prepared that he cannot use the simplest dichotomic table or recognise a single species.

A properly planned course in systematic entomology would do much to remedy this lamentable state of affairs. It would benefit equally the student looking forward to a career in applied work and one intending to devote himself to more purely taxonomic studies. The practical man might learn to see with the systematist's eye. He would go to the field at least able to detect specific differences between two insects even if he never attempted to name them. That in itself would be a great gain. It might save him the disappointment of being told that, after working up a life-history, *e.g.*, that his material comprised three species of two different families, and it might make unnecessary a great deal of speculation. The females in a certain group of scale insects were long asserted to be seasonally dimorphic and a theory was advanced that this dimorphism was due to retarded metabolism in the winter form. The plain fact was that authors of the theory had failed to discriminate between two or even more species living on the same plant.

It is then a sound instinct that prompts the question "What is it?" Without a name for his beast the applied biologist is helpless. It is only through the name supplied by the systematist that the results of research become communicable to others. This is the first and the most important help that the systematist can give you. But the service does not end here. Provided that the synonymy has been well studied, possession of the name gives access to all that has been ascertained about your species. And here I cannot refrain from saying a word in defence of the

systematist "who is always changing the names." For nothing is he more heartily or more undeservedly castigated than for this. Yet it must be clear that if investigation has proved that a species described in 1900 appeared under an older name in 1859, the general student knowing only the later name will overlook all the information that has been gained about the species in the interval. The specialist of course will not be misled. By forcing a change of name you compel the student of life-history or variation to acquaint himself with all that has been written on the organisms he investigates. In practice this may be a mixed blessing but the principle is a sound one. An adequate synonymy means a full background for your picture, and as the biologist is frequently so placed that he has not access to literature or collections, the spade-work of hunting up references has to be done for him and this again very often falls on the shoulders of the patient systematist. Nor does his usefulness end in providing names and references. When nothing has been recorded of the behaviour of a species just determined the systematist may be able to give invaluable pointers to the field worker, *e.g.* he may know that all the congeners of a parasite submitted to him are hyperparasites, or that, if genuine parasites, their control is not usually an effective one. He may be able to say that a particular pest is probably introduced and to predict where suitable controlling agents should be looked for. He can probably lessen his confrère's task by indicating the most profitable order for his enquiries and may obviate many an irrelevant excursion.

His work is thus fundamental and indispensable. He is the alphabet maker supplying symbols of articulate speech; the grammarian formulating its laws, the digger of the foundations, the maker of the bricks for any building that may be accomplished and the more clearly this is realised the greater will be the help that the systematist can give. It is by an ever closer co-operation between the two branches that the problems of both are to be solved and it may not be amiss if I indicate some of the ways in which the applied worker can ease the burden of the systematist and at the same time get the maximum benefit from his services.

You should state your problem as succinctly as is possible in consulting the systematist but at the same time quite unreservedly. I cannot conceive of any circumstances in which mystery making would be justifiable but I have known men who wanted "just the name" go on to find out facts already ascertained or to demonstrate what had already been experimentally established.

Again, while it is impossible to say, especially at the beginning of a piece of research, what organism or factor may be of importance, one can at least avoid conscious irrelevance. Do not include with your strictly research material specimens casually acquired. If you are working on the pests of a particular crop do not send in everything you come across in the hope that "it may be of interest." Very likely it will not.

Label your specimens carefully but first ascertain from your consultant what special notes he wishes to have. Place, date, host plant, relations to host are usually sufficient.

As to the condition of the material, you must again find out from the systematist how he wants it sent. Most systematists prefer to do their own preparatorial work.

It is by attending to these and similar common-sense precautions that you will enable the systematist to work most effectively and speedily and so to co-operate with you in making two blades of grass grow where one grew before or in ensuring that man and not the insect shall increase and occupy the earth.

Dr A. W. Hill (Royal Botanic Gardens, Kew), in discussing the necessity of systematic work referred to certain economic problems where the need of a systematic study of the plants yielding some particular product of economic value was of the utmost importance.

He instanced among other matters the West African oil palm (*Elaeis guineensis*), which extends from Sierra Leone along the West Coast to Central Africa and shows a succession of well-marked varieties and in some cases of species in the course of its range.

Some of these forms yield a better type and more abundant quantity of oil, either from the pericarp or from the kernel, than do others. In some cases an oil of a particular chemical composition is more favoured by the merchant at home than another. It is also found that the thickness of the shell, an important matter from the point of view of providing machinery, varies considerably in different varieties, in one or two the hard shell being reduced to a few fibres.

As it is found that the varieties yielding different types and quantities of oil and different thicknesses of shell can be differentiated without difficulty by the systematic botanist—and in fact have been so separated by the late Prof. Beccari—it is evident that whenever plantations of oil palm are to be formed the help of the systematic botanist must be sought if uniformity in type is required.

Dr A. D. Imms (Rothamsted Experimental Station) stated that he regarded the function of the systematist as being to identify, describe and classify organisms. Since it is impossible to deal with the vast array of known organisms unless they are named and classified, the position occupied by the systematist is one of great importance. In fact he would go so far as to say that the systematist is the man who provides the foundation upon which the whole structure of applied biology rests.

We are all acquainted with Shakespeare's views on nomenclature when he wrote "what's in a name"! Dr Imms added that he could not help feeling that if the immortal bard could have had the advantage of discussing the subject with Dr Waterston he might have written something very different! To us economic entomologists there is a great deal in a name, in fact our difficulty is that we so often do not know what name to use and we have to appeal to the systematist to keep us on the right track.

Dr Imms quoted several examples illustrating the great importance of correct descriptions and identifications in applied biology. In the case of mosquitoes carrying the malaria parasite correctness of identification is of paramount importance, since the behaviour of Anophelines differs greatly among themselves and control measures have to be adapted accordingly. With the Tsetse flies only certain species are at present implicated in the spread of sleeping sickness. Their identification is difficult, yet so much depends upon its being correctly carried out. We have the case of the sugar cane leaf hopper, *Perkinsiella saccharicida*, which, at one time, menaced the whole sugar industry of the Hawaiian Islands. On one plantation alone it reduced production from 19,000 to 7000 tons in three years. The entomologists of the Hawaiian Planters' Association were set the task of finding whence the insect came. It was ultimately traced to Queensland and, since an expedition was sent to that country to study the insect and its parasites in their natural environment, it is obvious how much depended upon its correct identification in the first instance—by

no means an easy task with an obscure insect with numerous closely allied forms. Also, how much misleading information would have been avoided respecting hibernation of the house fly if species found hibernating, and reported as *Musca domestica*, had been correctly identified!

It is significant to note that during the past five years nearly 250,000 insects from various countries were dealt with by the Imperial Bureau of Entomology and close upon 30,000 specific identifications made. This fact alone speaks eloquently of the demand there exists for the systematist in one single branch of applied biology since the above material came from men directly or indirectly concerned with economic work.

The most important service the systematist can do for the applied biologist is the adequate description of species. Of recent years this task has become immeasurably heavier owing to the fact that it is now realised that specific and generic descriptions have to be made with much greater detail and accuracy than hitherto. The earlier described species need re-description particularly in the light of the rapid discovery of new and closely allied forms.

Changes in nomenclature are now so rapid and confusing that the applied biologist can no longer keep pace with them. It has become more and more imperative that the systematist should provide revisions of at least the more important groups, collecting in a convenient compass the taxonomic changes that are only known through numerous scattered notes and papers.

We are, furthermore, greatly in need of more synonymic lists of the recorded insect faunas of different countries, especially of the British Isles and of various British dominions. The economic worker needs to know whether a given insect occurs in a given country, and if so, what is its correct name and its chief synonyms. In very few orders is there an up-to-date list of even the British species.

Another work systematists are better able to carry out than anyone else is the compilation of a host catalogue of each order of insects with its recorded parasites. The labour attending such a production would be enormous but its value would be undisputed.

Lastly we need a new type of systematist—men who will specialise upon larval forms describing them with the same care as is devoted to the perfect insects. Until monographs on larval forms are available for their identification, we shall be forced, in the greater number of cases, into either attempting to rear them to maturity or leaving them unidentified specifically. Dr Imms added that he knew of few greater technical requirements in economic entomology than accurate larval descriptions such as Dr Böving had given, which are a model for all who work at Coleoptera.

There is, however, a need to-day for systematists of broader outlook: many do not attempt to keep up to date with the progress of their science in other directions. There is a very great need for the quantitative study of the range of variation of the characters used for descriptive purposes. This fact is well exemplified by the Tachinid fly, *Exorista vulgaris*, which occupied $5\frac{1}{2}$ pages of synonymy in the *Catalogue of Palaearctic Diptera*. The characters which, in this species, are regarded as specific are so variable that worker after worker who has had the insect before him has described it as new.

Dr Imms stated that during a recent visit to the U.S. Bureau of Entomology at Washington, he was impressed by the fact that in the attempts to solve economic problems every effort is being made to probe deeper into basic principles than hitherto.

Correspondingly less reliance is being placed on the superficial knowledge that so often obtains in the hasty search for relief measures. This change of perspective is reflected both in the organisation of the Bureau and in the character of the work being carried out. The value of accurate specific descriptions and identifications, along with improved taxonomy, to the activities of the Bureau has recently met with full recognition in the establishment of a separate division of taxonomic entomology. In this division are listed some 16 entomologists, a number of whom bear names familiar in their own fields.

Mr A. D. Cotton (Royal Botanic Gardens, Kew) briefly emphasised the fundamental nature of systematic work to all other branches of botany and proceeded to deal with some of the charges brought against the systematist and with the question of recruitment.

In reply to the criticism that taxonomy is narrow and not scientific it can be said that there is plenty of scope for a wide outlook, and that descriptive work should be exact and its deductions accurate though, since it is essential that classifications should be in the first place intelligible and convenient, the arrangement may be artificial. It has further been said that for accurate systematic work the plants must be grown under controlled conditions. The worker with micro fungi acutely realises this, and it is the ideal, but is at present only possible in a few groups. To the controlled cultures (whether in the laboratory or field plot), studies in the natural habitat, and the historical side as represented by the Library should be added. The criticism of "bad" species was natural, though in view of the vastness and complexity of taxonomic work such errors were excusable.

Young systematists were required, especially for the dominions and colonies. The objections alluded to above should stimulate the true scientific worker to take up a subject which is fundamental to pure and applied botany and reaches out and affects questions of vast economic and political importance. In many groups, especially in the case of older countries, confusion as to species was great and intensive work under controlled conditions was essential. The time for the narrow type of systematist was passing and there was need of students with more modern outlook. Genetics and the experimental plot were needed on the intensive side, whilst on the extensive side there was ample scope with such problems as plant distribution, origin of floras and plant geography.

Dr Butler (Imperial Bureau of Mycology) drew attention to the neglect of the systematic study of fungi, as compared with that of the higher plants, in the overseas parts of the Empire. Whereas the study of the flowering plants was carried on in a systematic manner by the staffs of numerous Botanic Gardens, Herbaria and Museums in the Dominions and Colonies, that of the fungi had been largely centred at the Kew Herbarium and the Natural History Museum in London. There were, however, prominent exceptions to this general rule, such as South Africa, Ceylon, and New Zealand, where Government mycologists had organised the systematic survey of the fungus flora with the result that, as regards Ceylon, the known fungi of that small sland exceeded considerably those of the continent of India. The provision made by governments for the study of insects, inadequate as it evidently was in the opinion of those competent to judge, was still greatly superior to that applied to systematic mycology, possibly because, while the insects were recognised as a respectable group

of the animals, it was not always realised that the fungi were plants, occupying a considerable place in the plant world, and as such requiring to be investigated from the purely botanical point of view.

Instances of the serious economic consequences of this neglect of botanical studies of the fungi could be multiplied, under various aspects, especially when the plant parasites were in question. The same parasite has been believed to be a different species in different areas, with the result that intensive work on it in one area was not made use of in another. Different fungi attacking a crop have been thought to be the same, with consequent failure of methods of combating a disease that appeared to have proved successful elsewhere. The same parasite may have a dozen different names on as many different kinds of host plants, and its destructive possibilities may be correspondingly under-estimated. Common saprophytes of a region may be thought to be harmful and, conversely, really dangerous species may be ignored. Examples under these various heads were given, showing how they reacted adversely on the work of the plant pathologist.

Such cases showed that provision for systematic studies is an essential part of any adequate economic mycological organisation, that the systematist had a definite place in plant pathology, that no branch of systematic study could safely be neglected, and that it was useful for the general pathologist to have a sufficient systematic equipment to be able to supply the specialist in any group with adequate material for study.

Dr Rendle (British Museum: Nat. Hist.) emphasised the importance of a name as a mark of identity. The systematist cannot wait for perfect knowledge of an organism before describing it, and names are admittedly often assigned on the basis of imperfect material, the systematist must use his discretion; with increase of knowledge the description may be revised and amended or enlarged. In identification of specimens it is above all things important to have the right name, especially when economic use is concerned. The systematist must therefore be well equipped and well trained; it is bad economy to entrust work of determination and classification to unskilled or poorly trained workers. Hence it is important to secure the best men (or women) for the work, provided always that the required type of mind is present. Teachers should be encouraged to give not only the good general training in science, which is imperative, but also to instil a decent elementary knowledge of systematic work.

Dr Goodey (London School of Tropical Medicine) said:

I wish to say a few words on the helminthological aspect of this subject and perhaps before I go any further I ought to make a few remarks in explanation of the word Helminthology itself. Some of you may not have heard it before but I can assure you that it is quite an old word and the science of helminthology is quite an old one; volumes were being published on it more than a hundred years ago. It is the study of the Helminthes or parasitic worms of man, animals and plants. These can be split into two great subdivisions which are quite unrelated to each other, namely, the Flatworms, that is Tapeworms and Flukes, and the Roundworms or Nematodes. Knowledge of one of these great subdivisions is not by any means applicable to the other subdivision. The word Helminthes is a convenient collective one which has a useful faunistic meaning.

Then by way of preliminary also I ought to say something on the word systematist. If by systematist is meant one whose business it is to attempt to arrange and classify organisms into various groups and categories, and this is what I take the word to connote in a somewhat narrow sense perhaps, then I would say that Helminthology to-day and the applied biologist in Helminthology have very little use for him.

If, however, the word systematist is used to embrace the comparative anatomist and morphologist, who, in passing, must be somewhat of a draughtsman, then I would say that Helminthology and the applied helminthologist have a great deal of use for him.

Helminthology has suffered during the last 100 years from the attentions of the systematist, using the word in its restricted sense, and is to-day only on the road to a better, sounder, and a more natural scientific classification of the organisms with which it deals. This probably needs a certain amount of explanation, but the story is fairly simple and hangs on the influence and prestige which a great man can exert in his subject and also no doubt on the fact that man is naturally a conservative animal, disliking change and disturbance.

The first great classification of the Helminthes was made by Rudolphi in 1819. This remained a "standard work" for many years and still is of great importance. It led to stagnation in Helminthology in that it checked the scientific classification of parasitic worms advancing beyond that propounded by Rudolphi.

Confining our attention to the Roundworms, we find that Rudolphi collected all the species known to him into 11 genera, some of which even in 1819 contained more than 100 species. The majority of the species described since his time were forced into his genera and only when some peculiar outstanding difference presented itself was a new genus created for its reception. Thus it happens that if one looks up a list of some of the well-known genera, such as *Ascaris*, *Oxyuris*, *Heterakis*, *Strongylus*, *Filaria* and *Spiroptera* one finds hundreds of species under each genus and it is quite impossible to arrange these separate species as independent members of their genus in any really satisfactory and scientific manner. The fact is, these old genera were erected on the basis of one or several *external* characters; *e.g. Ascaris* principally on the possession of three lips round the mouth, *Oxyuris* on the shape of the oesophagus, *Strongylus* on the fact that the tail of the male is modified to form a copulatory bursa and *Filaria* on its general shape and simple mouth. These characters are of course valid and valuable but they do not carry us far enough. We want more detailed knowledge of the internal structure and organisation of all the worms and such knowledge is a prerequisite for the establishment of a satisfactory natural classification of the Helminthes which shall be in any way comparable and equivalent to say the classification of the mammals.

The older helminthologists apparently believed in the instability of the species and thought that it might vary within wide limits and once a worm had been found in a given host, when worms were again found in this animal, even though they only resembled the previously found parasite in one or two characters, they were placed in the same genus as it. This doctrine of the instability of the species had other consequences, for if a worm could vary in structure in the same host it was only natural to suppose that it should show other variations when found in other animals. It was also held that the worm was capable of living in and adapting itself to *various* hosts and though there is a certain amount of truth in this, it is more the exception than the rule.

To-day we recognise that the Nematodes, just as the higher animals, mammals, etc., possess constant specific characters which we may speak of as fixed and what is still needed at the present time is more and more knowledge of these specific characters. Systematisation of the various groups of Helminthes can come later when we have made as complete a survey as possible of the field. We are only now approaching the point at which such a classification according to their natural relationships can be attempted.

This study of specific characters is of course very largely a morphological one and for the better elucidation of his subject the worker should contrive to illustrate his writings with suitable drawings, which, if good and clear, can take the place of long descriptions. Measurements of important regions are valuable also but good drawings are often of far more use than tables of measurements without drawings.

The applied biologist in Helminthology has to be, to a large extent, a morphologist. He must know in detail the organism with which he is working and of course its near and more remote relatives and this brings him into contact with the systematic side of his subject. But in so far as he is mainly concerned with the applied aspect, he can have little time for its more purely systematic bearing and he may have to leave this to other experts whose sphere of work is not the applied side of the subject.

It may be of course that if the worker is dealing with a special group of organisms, say a disease-producing group, he will become *the* expert on them and consequently pre-eminently the person to speak authoritatively on their systematic arrangement. On the whole, however, I would say that the systematist in Helminthology, if he is merely an arranger and classifier and not at the same time a sound morphologist has little useful to offer the applied biologist.

The work of the latter in Helminthology is the study of the morphology, life-history, biology and pathology of parasitic worms and in addition, the search for the vulnerable spots at which they may be attacked by destructive or remediable measures.

As one whose work is applied and not purely academic he must tend to concentrate on the practical aspect of his study which after all is the living experimental side and leave the systematic arranging, listing and classifying of the organisms to those who have a special bent and opportunity to pursue their bent. Of course he may have bent and opportunity himself; the two sides are not mutually exclusive. I cannot, however, recall any great advance in applied Helminthology which has sprung from the more formal and systematic study of the organisms embraced by this word. The advances have come from the application of comparative morphological and anatomical studies and have been achieved by the men who have been trying to work out practically by experimental methods, the problems of the life-history and biology of the parasitic worms.

Dr K. Jordan (Tring Museum) said:

It is evident from what we have heard that, in the opinion of those who have spoken at this meeting, there is something amiss in the relation between systematics and applied biology. The student of applied biology requires information from the systematist which the systematist cannot fully give. This question, as far as it concerns entomology pure and applied, has been discussed last summer at two important meetings: in London at the Imperial Conference of Entomologists, and

at Zürich at the International Congress of Entomology. It was the general opinion at these meetings that the systematists in entomology could not possibly cope with the enormous demands on them, and resolutions were passed giving expression to a desire for amelioration of this most unsatisfactory state. The resolutions adopted at Zürich read as follows:

I. Resolution. Horn—Escherich—Nuttall.

The Section II for Systematics and Geographical Distribution having had under consideration the relation of Systematics to Applied Entomology wish to record the following observations and recommendations:

Observations.

1. An enormous expansion in Applied Entomology has recently taken place throughout the world. This has necessitated, as a first step, the exact determination of an immense number of insects. The result has been to show the utter inadequacy of the present means for undertaking such work.

2. While the existing organisation of Applied Entomology is efficient in some countries (notably in the United States and Great Britain), the number of trained workers available in all countries is generally recognised as totally insufficient. Everywhere the systematic specialist is over-burdened, and his load has now become an intolerable one.

3. Neither Systematic nor Applied Entomology has yet received due recognition in academic circles. Entomology has long been the Cinderella of the Sciences. Entomological work, especially Systematic Entomology, has been regarded as being definitely inferior in quality to work in other branches of Zoology. It is within the knowledge of the framers of this resolution that postgraduate students have been advised not to undertake research in Systematic Entomology on the ground that it led nowhere. At few, if any, European Universities is Systematic Entomology accepted as a qualification for a degree.

4. Such facts constitute a grave danger to science, both pure and applied, and, as a remedy for this state of affairs, the Section II proposes the following recommendations:

Recommendations.

A. With regard to Universities:

1. That the teaching of Systematic Entomology at Universities be adequately provided for by the establishment of chairs especially devoted thereto.

2. That meritorious systematic work should be regarded as qualifying candidates for ordinary and advanced degrees at Universities.

B. With regard to Museums:

3. That immediate steps should be taken to increase the number of entomological assistants, both systematic and technical, in Museums and that this should be done in a manner proportionate to the amount of specific work entailed.

4. That in the appointment of persons to administrative posts in Museums and like institutions due regard, *inter alia*, should be given to a candidate's experience in systematics.

C. With regard to Bibliographical Institutions:

5. That each country should have a central Bibliographical Institute (Library) for Entomology.

This Resolution, framed by W. Horn, K. Escherich and G. H. F. Nuttall, and in addition signed by C. Börner, J. Davidson, H. Eidmann, K. Eckstein, K. Jordan, R. Kleine, O. Morgenthaler, A. Schuberg, M. Schwartz, F. Stellwaag, J. Waterston and C. B. Williams, was unanimously agreed to at the General Meeting of the Congress on July 25th.

II. Resolution proposed by the Section V for Applied Entomology.

This Congress considers it essential that the problems underlying Applied Entomology should be studied, and desires to impress upon Governments and Institutions concerned with Applied Entomology that time must be devoted to Systematic Entomology and fundamental research such as Insect Physiology, Ecology and Pathology, since only by the study of these can insect control be placed on a sound basis.

This Resolution, signed by C. Bolivar Pieltain (Spain), C. Börner (Germany), J. Davidson (England), T. Bainbrigge Fletcher (India), L. O. Howard (U.S.A.), J. Jablonowski (Hungary), R. Stewart MacDougall (England), G. A. K. Marshall (England), J. W. Munro (England), O. Schneider-Orelli (Switzerland), F. Schoevers (Holland), F. Stellwaag (Germany), W. R. Thompson (U.S.A.), I. Trägardh (Sweden), F. W. Urich (Trinidad), C. B. Williams (Egypt), was unanimously agreed to at the General Meeting of the Congress on July 25th.

These resolutions have been sent to the authorities concerned, and now the question arises as to whether we are satisfied with having expressed these opinions or whether we wish to see the recommendations carried into effect. There will be no adequate result unless the resolutions are driven home by united action of those who know the enormous economic importance of the questions involved, and this Association would greatly assist the efforts to be made by expressing agreement with the Zürich resolutions. I therefore place before you the following resolution:

"That this meeting of the Association of British Economic Biologists strongly supports the recommendations as to Systematics embodied in the resolutions unanimously passed by the Third International Congress of Entomology (Zürich) at the General Meeting on July 25th, 1925."

For various practical reasons it is advisable to restrict, for the present, the demands for amelioration to Entomology, a field of research where the great needs of Hygiene, Agriculture, Horticulture and Forestry throughout the Empire are so obvious and so very pressing. In order to achieve positive results in the nearer and farther future, a plan of procedure should be worked out. I suggest that a small Committee, which could conveniently meet in London from time to time, should take the matter in hand, keep the agitation alive and enlighten the public, and I hope that the Council of this Association will appoint a member for that Committee. The Committee should be quite clear about the points which require speedy amelioration in Systematic Entomology and also must consider the various kinds of opposition it is *a priori* certain to encounter. In this campaign—if I may call it so—the Committee will have on its side a great asset in the fact that it will work for no self-seeking

purposes, but for the enhancement of the efficiency of Applied Entomology—which depends to a great extent on Systematics—and thereby will contribute greatly to the betterment of the economic conditions in the Empire.

Mr J. Ramsbottom (British Museum: Nat. Hist.) said:

The place of the systematist in applied biology seems to be that which he should occupy in all biology. It is a feature of much modern work that the last matter with which an investigator occupies himself is the name of the organism he is studying. When the research is concerned with phytopathology it seems reasonable to suppose that the first thing would be to obtain an accurate identification of the parasitic organism, for when this is known the way is open to make use of the results of previous investigators if such there be. It is not unusual to have a specimen brought when the account is being written up. Systematists are supposed to appear in public in sackcloth and ashes and to bear one another's burdens in a way not expected of physiologists, cytologists—or even plant pathologists. I am not concerned with inferior systematic work—there is bad systematy as well as bad physiology. Is there not a common misunderstanding of what a systematist sets out to do? On the theoretical side he attempts to arrange the organisms which he is studying in a natural system. For this purpose any fact discovered about an organism may be of use, and these may be obtained from morphology, anatomy, physiology, cytology, genetics, etc. A natural system is a pious hope and most systematists do not publish an attempt at such but adopt already existing efforts with mental reservations. In mycology we are in a much better position than in botany in general. We are in this position because most of our ideas seem to be in a state of flux. The intensive study of parasitic organisms which many plant pathologists have been compelled to make, though its immediate effect has been to curse the systematist and all his works in reality is resulting in great additions to the tools with which systematic mycology has to deal. In our present state the systematist should not be looked to for detailed investigation of some odd genus but rather as one who sifts the heterogeneous mass of facts presented to him for clues as to relations. On the practical side there is the identification of organisms. A systematist has to attempt the naming of individual specimens and it is this portion of his work which often produces the rub. Economic workers sometimes fail to realise that an odd culture may in no wise resemble the original description of the species; *e.g.* a non-sporing mycelium of *Fomes igniarius* on malt extract agar in a test-tube has quite a different appearance from the fruit body a foot across on a tree-trunk. But as our description becomes more detailed it should include an account of such mycelium and an identification could be made. The "new species" of a systematist simply means that he cannot find a description to match the organism: what economic biology has to guard against is that all its organisms should not be so labelled. Dr Butler spoke of the percentage of species of fungi still to be discovered in the East. The number of species still to be described in the country is probably 50 per cent. of the whole.

Mr Cotton spoke of systematic botany as a career. But in mycology in this country there are only three people with definite systematic posts—and none of us very aged. This brings me to the motion put forward by Dr Jordan. Nothing can be done unless the Universities are persuaded that systematic work is worthy of some amount of trouble. So far as I am aware there is not even a lectureship in sys-

tematic mycology in any of our Universities nor any lectures for the many post-graduate students desiring them. It is my opinion that the attitude of the schools towards systematic botany in general makes possible the present discussion. To go no further than London Univesity. A hundred yards or so away from this building there is the British Museum (Natural History) with a very large scientific staff, many with an international reputation and the majority honours men in their University. Some serve on different Boards of Studies but so far as I know not one of them is ever invited by the University or any of its constituent colleges to give lectures on their subjects. It would be out of place for me to develop the idea here but it is one that appears worthy of consideration.

It would be interesting to know how far modern botanical teaching has tended to excite an interest in systematics. The apparent result would seem to indicate that the ideas prevalent a few years ago are still predominant. Does a student never wish to do systematic work for a research degree—and, if so, what happens? It appears strange to me that students should go through honours schools in the numbers they do and then go out of botany. Is it not a fact that the majority of such students have so been trained that they would be more or less competent to do a piece of research in a well-equipped laboratory but that they have so little idea of systematic botany that they do not know the books to use to identify the common cryptogams of the country?

Dr Brierley (Rothamsted Experimental Station) said:

From the remarks of previous speakers it has not been easy to gain a precise notion of what a systematist really is or does, but it may be that these things vary in different fields of biology. We are all systematisers in that we arrange and classify ideas and data and, if these data characterise groups of living things, we become systematists. I think, however, that our issue to-day is not the systematist in this more general sense but the systematist in the special and narrow sense—the man occupying a professional systematic post in a herbarium, museum, etc., or the amateur doing similar work. Further, I think our discussion really concerns the place in applied biology of present-day systematics and not of some ideal state of things; if we stray from what is and discuss what might be, we shall lose touch with reality and take refuge in a variety of Utopias.

My own experience of the systematist (but this may be partial) is that he is one who assembles specimens, writes diagnoses of them which he arranges in convenient or other systems and publishes with or without keys so that specimens of similar morphology may be given the same name—in essence he is one who classifies and gives names to specimens. No one can doubt the value of such systematology and, in my own field of work, I can say quite straightly that without our Rabenhorsts and Saccardos, Kews and Natural History Museums many aspects of mycology would not exist. But, the more successful the systematist is, the less necessary does he, personally, become, for once his diagnoses and keys are printed other people can use them as well as he can and themselves classify and name their specimens. The systematist, in fact, works towards his own elimination and should rather hold it a source of pride than regret that he no longer occupies the same pre-eminent position as in the younger days of biology. Of course the pioneer and the rare man gifted with the *systematische Blick* will always be eminent. The meticulous exactitude,

rigid nature and authoritarian formalism of systematology, which are the rightful boast of the systematist, are also the reasons why the "bad systematy" to which Mr Ramsbottom has drawn attention is very much worse than bad physiology. A name is a defined end objective, a thing standing alone and the ideal of the systematist is to eliminate the personal equation in the attainment of that objective. The better the systematic work the nearer is this ideal approached. Further, in systematic studies, there is no room for calculation of averages, standard deviations, etc., as in physiology: one cannot calculate the degree of badness of a specific determination.

Criticism of the systematist is usually directed against his conservatism, his narrow outlook and his lack of contact with the more living or, at least, the more experimental aspects of biology. Some part of this criticism can hardly fail of justification for the systematist is usually immured in a herbarium or museum with few or no experimental facilities, he is often overburdened with routine work, he breathes an atmosphere of old books and dead things and splits hairs in a dead language, all his traditions are armoured in morphological rigidity and stiff with respect for authority and the claims of priority. Under such conditions it would need a superman to retain any lively interest or intimate personal contact with the overwhelming stream of experimental research in biology. It would be remarkable, indeed, if the values and points of view of the systematist were not often considerably out of perspective in applied biology.

Fortunately for mycological systematics the sheer pressure of plant pathology is rapidly bringing about a fundamental change, a *rapprochement* between the systematist and the applied biologist. The plant-pathological or behaviourist viewpoint is being grafted on the well-rooted stock of morphological formalism and laboratory facilities are being added to herbaria so that the systematist can assimilate the physiological criteria of the experimentalist. As Mr Ramsbottom has well said our ideas, and I would add our practice, are in a state of flux and this is a most hopeful sign for the future.

Of course the present difficulties are great and the applied biologist, confronted by his practical problems, is at times apt to be impatient with the systematist and a little sceptical and suspicious of his artificial values. My own pure faith has never quite recovered from an early experience when duplicate cultures sent to a well-known systematist for identification were placed in different genera. However, in spite of such rare accidents, every plant pathologist and indeed every applied biologist realises full well his indebtedness to the systematist whose labours have produced some semblance of order out of chaos and enabled the experimentalist to speak familiarly of the organisms he studies.

Systematics is in fact an essential tool in applied biology. Like all tools, however, it must be used only for its special and legitimate purposes and kept in its proper place. If it occupies less than its true niche in the structure of applied biology or if it occupies more, or if it is used for wrong purposes, there will result waste of energy, confused thinking and a wrong balance of values. I cannot help feeling that certain previous speakers in their enthusiasm for systematics have claimed for it rather more than its due comparative share of importance. Personally, I see systematics merely as one weapon among many in the armoury of applied biology and the relative importance of these weapons depends entirely upon the nature of the problem to

be attacked. To the applied biologist these weapons are not ends in themselves but means to an end and he is prepared to use each or all of them according to the service they can render. There are whole series of problems in applied biology in which systematics must be the first weapon to hand and equally important series of problems in the solving of which it plays little or no part.

It is, however, of vital importance that when the systematic weapon is needed it should be there and that, confronted with our problem, we should not then have to begin searching for our weapon or in desperation try to forge one anew. This is our weak point. In mycology (which is an important aspect of the study of plant disease and disease in plants is one of the primary controlling factors in crop growth) we have, in this country, practically no mechanism for training students in the classification of fungi or for retaining them as systematists if trained. So far as I am aware there is no Chair of Mycology in any University in the British Empire and Mr Ramsbottom states that in this country there are only three people with definite posts in systematic mycology. When one considers, even by purely utilitarian standards, the sheer importance of fungi in agriculture, in industry and in other vital branches of human welfare, this condition of things is amazing. I do not know whether entomology is more or less important than mycology, it is a nice point of debate, but there can be no question whatever that almost everything that has been said by previous speakers regarding the needs of entomology and entomological systematics can be repeated of mycology and mycological systematics with even greater urgency and emphasis.

Mr R. Stenton (Pathological Laboratory, Ministry of Agriculture) said:

I speak as one of that long queue spoken of by Dr Waterston as asking "What is this thing"? Nearly all the previous speakers have been systematists in one sense or another of the term so there is no need to say more along the lines already followed.

Dr Waterston has hinted at castigation, Dr Jordan going further and saying that something is wrong. With the fullest possible appreciation of the work done by systematists and appreciation of their position being a fundamental one, I think too that something is wrong, and that purely biological workers have cause for complaint as to details of systematic work. Systematists have given as their function the giving of the correct name to an organism, but it appears to the biologist that they can never decide on what is the correct name. The movement to straighten out the synonymy appears to the harassed biologist to have resulted in rendering the confusion more confounded.

It is not, however, only in synonymy where difficulties are encountered: systematic work is not always presented in an easy form for use. There should be, with adequate descriptions and tables, no need for that long queue asking "What is this thing"? As matters stand, it requires many years' application to some orders of insects before venture can be made to identify a specimen from the standard text-book on the subject, at least with any feeling of certainty.

It may be asked—with what object do systematists set to work? Is it to obtain an arrangement in accordance with the natural evolutionary procedure, with the hope that also the work may be useful to biological and other workers? Any arrangement as carried out on the approved systematic lines is admittedly artificial and approximate only to the natural course, while users of systematic work complain

much of its imperfections—so perhaps the systematists in setting out to please everybody, succeed in not entirely pleasing anybody.

With the ever increasing numbers of biological workers some attention might be given to turning out better systematic work in the sense of presenting it in forms more directly useful to the biological worker. There is one standard entomological work at least from which I believe no insect has ever yet been identified.

Referring again to synonymy, there is one point worth mention—there is a large section of the public, agriculturists and horticulturists, who are making an increased use of the scientific names applied to the pests and diseases which concern them. To those whose business it is to digest scientific work and pass on the result in an easily assimilated form, the problem presented by ever-changing names has proved insoluble. Are the old familiar names to be retained at the risk of being accused of being unscientific and misleading, or if every change be adopted what explanation can be given to the practical man?

Mr Fryer (Pathological Laboratory, Ministry of Agriculture), after endorsing generally the remarks of other speakers in regard to the value of systematic work, said he desired specially to emphasise a point previously made by Dr Hill in regard to the desirability of giving economic biologists some training in the principles of taxonomy and in their practical application to the identification of organisms. It was realised that economic biologists could not become authorities in the systematic world but they could, and should, have enough knowledge of the subject to contribute to the common end. Definite training in systematic principles should be part of the equipment of the economic biologist. He needed such training first because he would often be working in regions in which he would be deprived of the assistance of the specialist. Then the ability to place an organism in its correct group would often yield invaluable clues as to its biology and the part it might be playing in the problem in hand. But it would also help in the following manner. Since systematists were compelled for most of their time to work near their herbaria and collections they could not often test by the study of the living organism the taxonomic value of the morphological features which they detected, but in this the field biologist could assist and co-operate. Even though a man's time was very fully occupied, he would often have the chance of gaining a specialised knowledge of a small group of species concerned in some economic problem—which knowledge would be of great assistance to the Museum systematist. As an instance, reference was made to a polymorphic species of stick insect which Mr Fryer had dealt with in Ceylon. In this case the progeny of one mother comprised, according to the systematists, three species and two genera, thus proving that some of the morphological features relied upon to distinguish species and genera in the Phasmids were of no value whatever. If the study of systematics were spread over the whole field of biology and not left solely to a specialised group of workers, some, at all events, of the difficulties referred to by previous speakers might be overcome.

Dr H. A. Baylis (British Museum: Nat. Hist.) said:

I should like to begin my remarks by putting one or two questions which seem to me rather pertinent to the subject under discussion. In the first place, is not the distinction between systematic and economic biologists rather an artificial one? Are not all biologists who are occupied with groups of so-called economic importance

doing economic work? And, for that matter, is it not rather arbitrary to label one group as of economic importance and another not? Can we ever safely make this distinction?

There is a tendency in some quarters to sneer at systematists, and certain people never use the word "systematist" without the implication of a sneer. This is probably because all the bother about names seems, to some people, a waste of time. Unfortunately it cannot be denied that there is a certain class of systematists, who ought rather to have a distinctive title such as nomenclaturists, who lay themselves open to this kind of criticism by seeming to care much more for the name than for the thing of which it is the symbol. But if we are honest we must all realise the usefulness of deciding what is the proper name of a species and then sticking to it. In no other way can hopeless confusion be avoided. But it is obvious, of course, that the thing that really matters is the definition of a species and not its name.

The President of Section D at the British Association this year gave an unusually candid definition of what he thinks should be understood by a species. He said, "A species is a community, or a number of related communities, whose distinctive morphological characters are, in the opinion of a competent systematist, sufficiently definite to entitle it, or them, to a specific name." Probably many of us have long wanted to know what a species is. Now we are assured by an eminent authority that it is merely what a competent systematist thinks it is, and perhaps we may have suspected this, though we hardly dared to say so. Anyhow, one sometimes feels that the time is approaching when it will have to be admitted that no hard and fast lines can be drawn between species. In my own branch of work, helminthology, the time seems indeed to have already come when the purely morphological definition of species is showing signs of inadequacy. We are being confronted, from time to time, with so-called "physiological species," or, as some prefer to call them, "host-races," which, while morphologically indistinguishable, nevertheless show well-marked differences in their adaptation to particular hosts, and in some cases are apparently incapable of being interchanged. If this kind of thing becomes general, it seems that the old-fashioned systematist is likely to have a bad time. It looks as if he will have to give up his favourite morphological ideas and become an experimental biologist. However, for the present there still seems to be plenty for him to do, and he may at least still be allowed to form his *opinions* as to what species are, basing these opinions, primarily at any rate, on morphological characters.

The important thing, and this is what I want to emphasise, is that there must, in any case, be people who are in a position to form opinions of this kind, and I venture humbly to submit that not all biologists are in that position. Systematic work and economic work, in the accepted sense, are both nowadays wholetime jobs, and therefore there must, for practical reasons, be a division of labour. In order to form his opinions the systematist must be able to keep in touch with all the vast and scattered literature of his subject, and he is obliged to pay attention not only to the morphological side of it, but to have at any rate a working knowledge of what is going on on the experimental and economic sides as well. He ought also to have access to adequate collections of specimens for reference and comparison. In other words, he requires both a complete library and a well-stocked museum. The so-called economic worker is very rarely fortunate enough to possess these facilities, or to possess the necessary knowledge and experience to use them rightly. He should

therefore refrain from sneering at the systematist as a kind of inferior being, but should regard him as a valued colleague who holds the key to a great deal of information that would be inaccessible without his help.

In this defence of the systematist I have said nothing about the wider scope of his researches in revealing the workings of evolution, but have been looking at him from the angle customary with workers in other fields, namely as a kind of mere machine for turning out the names of species. I hope nobody will imagine for a moment that that is the whole function of the best kind of systematist.

It is clear, then, that a division of labour is necessary, and that the systematic worker needs a very full equipment to enable him to work efficiently. It may be admitted that in these days the world cannot get on without the economic biologist. Very well, but let the world not forget that the economic biologist can scarcely begin his work without the help of the systematist, and certainly cannot continue it very far without constant reference to the literature to which the systematist, with his much-despised but necessary names, gives him the key. Let both workers, therefore, receive their due share of credit for the work accomplished. Instead of adopting superior airs towards the systematist, the field worker should show his appreciation of his assistance by helping him to form those complete collections which will constantly increase the systematist's efficiency, and will, in the long run, redound to his own advantage.

And this leads me to say a few words on a more personal note. One of the most important functions of the Natural History Museum is to assist economic workers by supplying the names of specimens, and all it asks in return for this service is to be able to keep part of the material when it is a desideratum to its collections. Speaking from my own experience in the helminthological section of the Zŏological Department, I would say that two points are apt to be forgotten by outside workers in sending material for identification. One is that the material should be preserved in such a way as to facilitate rather than hinder determination—the Museum issues a series of Instructions for Collectors, covering almost every kind of material, so that there need be no doubt as to the methods that are found satisfactory. The other point is that whenever possible enough material should be sent to enable a fair division to be made. In many cases to send a longer series of specimens would cost the sender no extra trouble, and it would help enormously in increasing the value of the national collection to the poor systematists who are trying to form their "opinions" about species.

Prof. J. H. Ashworth (University of Edinburgh) wrote that he was unable to be present but that he had referred to the value of morphology and of taxonomy in a recent address from which he quoted the following paragraphs:

There are some groups of animals, *e.g.* the earthworms, in regard to the evolutionary history of which we can never hope to receive help from palaeontology; we must perforce make the best use we can of the morphological method applied, be it understood, with wide knowledge and deep insight. That careful systematic work, coupled with the skilful application of sound morphological principles, is capable of yielding results of specific and general importance is well illustrated by the researches of Michaelsen and of Stephenson on Indian Oligochaetes; these authors have been able to trace the lines of evolution of the members of the family Megascolecidae so completely that we know their history as well as we know that of the Equidae.

The danger that morphology will be pushed to excess is long past; the peril seems to me to be rather in the opposite direction, *i.e.* that some of our students before passing on to research receive too little of that training and discipline in exact morphology by which alone they can be brought to appreciate how the components of the living organism are related to one another and to those of allied species or genera, and how they afford, with proper handling, many data for the evolutionist. I plead, therefore, for the retention of a sound and adequate basis of morphology in our zoological courses.

Another of the basal supports of our science an appreciation of which, or better still a training in some branch of which, we must encourage is the systematic or taxonomic aspect. The student or graduate who is proceeding to specialise in experimental zoology or in genetics particularly requires a sound appreciation of the fact that the accurate determination of the genus and species under investigation is a primary requisite for all critical work—it is part of the fundamental data of the experiment and is essential, if for nothing else, to permit subsequent observers to repeat and perhaps to extend any given series of observations. Moreover, the systematic position of an animal is an expression of the final summary of its morphology and its genetic relationships, and it is from such summaries that we have to attempt in many cases—as, for example, in the Oligochaetes already cited—to discover in a restricted group or order the probable course of evolution though the method of evolution may not be ascertainable. From these summaries prepared by systematists issue problems for the experimental evolutionist and the geneticist. As Mr Bateson has pointed out, it is from the systematist who has never lost the longing for the truth about evolution that the raw materials for genetical researches are to be drawn, and the separation of the laboratory men from the systematists imperils the work and the outlook of both.

The relation of fleas to plague provides one of the best and most recent illustrations of the necessity for careful work on the systematics and on the structure and bionomics of insects concerned in carrying pathogenic organisms. The distinction of *Xenopsylla cheopis* from *X. astia* is not an entomological refinement with purely systematic significance, but corresponds with a different relation of the species to the epidemiology of plague, and hence becomes a factor of great practical importance. If through these researches it has become possible by examination of the rat-fleas of a locality to estimate accurately its liability to plague, anti-plague measures may henceforward be restricted to those areas in which plague is likely to occur, *i.e.* where *cheopis* is the predominant flea.

In all work with human Entamoebae there is need for critical determination of the species, for, in addition to *E. histolytica* [the causal organism of amoebic dysentery] a closely similar species, *E. coli*, is a common inhabitant of the intestine. This however is a harmless commensal, feeding on bacteria and fragments derived from the host's food. The distinction between the two species rests chiefly upon the characters of the nuclei and of the mature cyst, quadrinucleate in *histolytica* and octonucleate in *coli*—and considerable care and technical skill are requisite in many cases before a diagnosis can be given. And yet this distinction is definitely necessary in practice, for indiscriminate treatment of persons with Entamoeba is indefensible; treatment is only for those with *histolytica*; it is useless for those with *coli*.

REVIEWS

Manual of Vegetable-Garden Diseases. By Charles Chupp, A.B., Ph.D. New York: The Macmillan Company, 1925. Pp. xxiv + 647. Price 4 dollars.

The literature on plant diseases has now become so extensive that general textbooks on the subject which had to suffice for pathologists and cultivators a generation or so ago do not meet present-day requirements. The tendency to deal with the diseases of the various classes of cultivated crops in special treatises is an inevitable one and needs no discouragement so long as the presentation of the principles and fundamentals of plant pathology in its more general aspects does not, at the same time, suffer neglect. The attempt to expand the old general textbook by the incorporation of new matter to make it cover modern needs has in one well-known instance been a signal failure, and the appearance of reliable pathological works with more circumscribed but withal specific aims is a step in advance.

Even with the crops raised in one branch of the agricultural or horticultural industry such as those derived from the modern vegetable or market garden, however, it is barely possible that a man—especially if he be a young man—should have had close personal investigational contact with each and all of the diseases to which the plants are heirs; and a book dealing with them must to a considerable extent be a compilation depending on the original work of others.

The author of the present Manual frankly realises this and modestly wonders whether his present effort may not be a case of rushing in where wiser heads would fear to enter. Let him have no regrets, for his book fills—and not inadequately fills—a gap and, on the whole, satisfactorily meets a distinct modern requirement.

In the first eighteen chapters the hosts are grouped, nearly related ones being considered together, and the various diseases are discussed systematically in regard to their symptoms, causes and methods of control. The last two chapters deal with soil sterilisation and fungicides respectively. In some publications emanating from the United States of America there is a tendency to indiscriminate illustration; the present work, however, does not suffer in this respect. The one hundred and fifty-five figures provided are mainly illustrative of the external symptoms of the various diseases; they appear to have been selected judiciously and are for the most part adequate and satisfactory. Mycological details of the parasites concerned are not dealt with to any extent, for the book is intended to appeal more to growers and to students of plant pathology than to the mycologist as such. An excellent feature of the book and one which will add much to its usefulness to the student consists in the short lists of references to original papers which are appended not in an unwieldy mass at the end of the book, but in the most appropriate positions, viz. immediately following the diseases discussed. The author is to be congratulated in having spread his net wide in this connection, and in having included important papers published in many other countries than his own, thus indicating that he has no narrow conception of his task.

Although some of the host plants included, such as Pepper, Sweet Potato, Okra and Egg Plant are not grown as crops in this country and their diseases will be of less interest on that account to workers here, yet most if not all of our own vegetable crops are dealt with and the Manual should prove of considerable value to students and growers here. To workers in the colonies and elsewhere the inclusion of such hosts and the diseases which attack them will be welcome and the book therefore makes an appeal to a wide circle.

So far as the present reviewer has been able to test it from his own knowledge of particular diseases, the information supplied is reliable and up to date. The style is clear and concise; one's eye may perhaps be offended by the reluctance to supply the "u" to "mould," and "Rhizoctoniose" is an ugly word! Happily, however, the book is largely free from those idiosyncrasies in phraseology and orthography which sometimes creep even into phytopathological literature, and which distract and annoy the educated reader. As already remarked, the book should prove valuable not only to the grower, but also to the student, be he still confined to school or college or have passed unscathed through these ordeals determined to remain a student for life.

G. H. PETHYBRIDGE.

Fungous Diseases of Fruit-Trees and their Remedial treatment. By G. H. CUNNINGHAM, B.Sc. Auckland, New Zealand: The Brett Printing and Publishing Company, Ltd., 1925. Pp. xxxi + 382.

The problem of educating the practical fruit-grower in the principles and details of plant pathology and plant hygiene is no easy one. To catch him young and put him through lecture and laboratory courses in conjunction with practical work in the orchard would appear to be the best method of procedure; but to-day, at any rate, those who can afford the time and the expense for such training are in the minority. The immediate problem therefore is the education of the adult, who, although he may be blessed with acute powers of observation and reasoning, yet lacks, as a rule, a sound training in scientific method and a knowledge of the fundamental principles of biology and other sciences.

The written or printed word must be, in such cases, the principal method of educational effort; but one sometimes almost shudders at the enormous masses of leaflets and brochures which the never-wearying press uninterruptedly showers upon us. True, authors are thus kept busy; but apart from this, one is sometimes tempted to pause and exclaim with Robert Browning's organist

"To what issue?
Where is our gain at the Two-bars?"

No such questioning, however, arises in one's mind in connection with the book under review. Many readers of the *Annals* will probably already be acquainted with the excellent articles on the diseases of fruit trees which have appeared from time to time during recent years from the pen of Mr Cunningham in the *New Zealand Journal of Agriculture*, and better work of its kind is not to be found anywhere. The present book is to some extent an outcome or elaboration of these articles and is divided into two main parts, a General Section and a Special Section. In the first part such things are dealt with as the geography and climate of New Zealand, the elementary botany of the fruit tree, wounds and their treatment, general notions concerning fungi and bacteria, pathology and remedial treatment. Four chapters are devoted to this last-named subject, and it is interesting to note from the preface that "no information regarding spray treatment is given, unless the treatment has been put successfully into operation." The special part consists of some eighteen chapters in which the principal fungous, bacterial and other diseases of the various classes of fruit trees are dealt with in detail, and keys for their diagnosis are supplied. The book, being mainly for the grower and not for the mycologist, rightly does not contain a mass of technical details concerning the parasites causing the various diseases. Sufficient is, however, said to enable a good grip of the essentials to be obtained which are necessary for recognition of the diseases and for an intelligent comprehension of the available methods of control.

The book is abundantly illustrated with one hundred and thirty-one photographic reproductions and fifty-five figures. For the most part these illustrations are extremely clear, the paper being of the "art" type suitable for half-tone block reproduction.

There is a bibliography of over three hundred items which includes, amongst others, all papers published in scientific periodicals in New Zealand dealing with fruit tree diseases; there is also a glossary of the more or less technical words employed.

Mr Cunningham has evidently been at considerable pains to ensure correctness and consistency in the matter of nomenclature, both of the diseases themselves and of the parasites which cause them, a subject to which by no means all writers on plant pathology pay sufficient attention.

By being hypercritical it would perhaps be possible to find in this book, covering as it does so much ground in such a comparatively compact and concise form, minor points of disagreement with the author, but any such criticism would in no way detract from the value of the book as a whole. Although written of course principally for a New Zealand *clientèle*, many of the diseases with which the book deals are of world-wide distribution; moreover the principles of plant pathology know no regional or climatic limitations. Hence the book deserves and will doubtless secure a wide circulation; no fruit-grower's or plant pathologist's library should be without it.

G. H. PETHYBRIDGE.

A Synopsis of the Families and Genera of Nematoda. By H. A. Baylis and R. Daubney. London: Printed by Order of the Trustees of the British Museum, 1926. Pp. xxxvi + 277. 10*s*. 6*d*. net.

The Nematoda or Roundworms are a group of organisms possessing certain morphological characters which separate them clearly from other worms. A large number of them are parasitic in habit and are the causative agents of disease in animals and plants. There are several important diseases of human beings caused by nematodes particularly in tropical and sub-tropical regions and it has been in the investigation of the life-history of these worms that some of the most interesting facts of biology have been revealed. The veterinarian and the helminthologist have long studied the many varied types of nematodes infecting domesticated and wild animals. In addition to causing specific diseases there can be no doubt that many of these worms are responsible for poorness of condition and general unthriftiness in farm stock and are consequently of considerable economic importance. Amongst cultivated plants certain important food crops are susceptible to attack from nematode parasites whilst others of importance to the horticulturist, such as the narcissus and the hyacinth, to mention only two, are also subject to serious diseases due to nematodes. The plant pathologist is constantly encountering some of the smaller members of the group known as eelworms which occur in decaying vegetable tissues and the problem of differentiating between those which are true parasites and those which are non-pathogenic, secondary invaders is sometimes a difficult business requiring a detailed knowledge of the minute anatomy of the worms concerned.

In common with that of most of the biological sciences the literature dealing with the Nematoda alone is of enormous proportions and is very scattered. Helminthologists, however, have for several years been under a deep obligation to the bibliographical labours of Stiles and Hassall who have brought out three Index Catalogues of Medical and Veterinary Zoology. The last of these appeared in 1920 and deals with the Nematoda. It gives references to all the genera and species of nematodes with the authors and dates of publication in every case. By means of it and the Authors' Catalogue by the same two authors one is enabled to run down an original paper on any nematode species. So far there has been no book giving the characters of each genus but this want has now been met by the volume under review. In a systematic index the group is split up into five orders which are again divided into families, sub-families and genera. Generic diagnoses of more than 600 genera are given but those described since 1923 are not dealt with. In the Introduction, p. ix, the authors explain the classification adopted and state that they have included under the Ascaroidea those forms having three lips or obvious modifications of three lips as they consider these to be primitive. This is surely only another way of saying that

they show evidence of triradial symmetry common to the vast majority of nematodes. They separate the Strongyloidea and the Filarioidea from the Ascaroidea on the possession of certain other special characters and consider that these orders are of collateral rank with the Ascaroidea though they admit that they still show some evidences of original triradial symmetry. We are not convinced of the soundness of the scheme proposed by the authors for it seems to us that on the grounds proposed, both the Strongyloidea and the Filarioidea could equally well be considered as sub-orders of the Ascaroidea as collaterals with it in that their members show quite as much evidence of the primitive triradial arrangement of structures as many of those families, sub-families and genera placed by the authors in the Ascaroidea.

Any diagnosis of a genus must be accepted with the reservation that it represents the author's concept of the genus and it is possible that workers here and there will take exception to some of the diagnoses in this book. The authors have taken a fairly conservative view of the genus *per se* and this we think is all to the good, for there has been too great a tendency in some quarters needlessly to split up well-defined genera into sub-genera and new genera. Accompanying each diagnosis is the name of the habitat, the genotype and a short list of the most important papers on the genus. In addition in some cases a brief discussion is also given on critical questions of nomenclature. The species of each genus are not dealt with, there are no illustrative figures neither is any account given of the numerous facts of great biological interest concerning the life-histories of many of the parasitic nematodes; three points which we think reduce the value of the book considerably to the general biologist. It is essentially a work for the specialist in helminthology who will no doubt be very glad to have so handy and compact a collection of descriptions of the very numerous genera of the Nematoda. The authors have done their work thoroughly and well and the printing and general arrangement of the volume are excellent.

T. GOODEY.

LAWS OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS

I. The Association shall be called "The Association of Economic Biologists."

II. The object of the Association shall be to promote the study and advancement of all branches of Biology with especial reference to their applied aspects.

III. The Association shall consist of Ordinary and Honorary Members.

IV. Each candidate for ordinary membership shall be a subject of the British Crown. The nomination form of each candidate for ordinary membership shall bear the signatures of two members and shall be forwarded to the Secretaries. The nomination shall be submitted to the Council and, if approved, the election of the candidate shall be recommended to the Association at the next General Meeting. For the election of any candidate two-thirds of the votes of the members present and voting shall be cast in favour of the candidate.

V. All ordinary members on first election shall pay an entrance fee of half-a-guinea. Ordinary members shall pay an annual subscription of twenty-five shillings, due on January 1st of each year, or may compound for their subscriptions by payment of a sum of twenty-five pounds.

VI. Every member elected to the Association shall receive notice to that effect from the Secretaries and shall continue a member until his written resignation shall be received by the Secretaries, or until his membership be forfeited under the laws. (A member shall be liable for the annual subscription for the year in which his resignation takes effect and, notwithstanding his resignation, shall, if he so desires, receive any subsequent publications of the Association issued during that year.)

VII. Ordinary members shall be entitled to admission to all the meetings of the Association, to vote thereat, to present papers, to take part in discussions, and to receive a copy of the Association's publications. Each member shall be entitled personally to introduce non-members to any General Meeting of the Association. But no member whose subscription is in arrears shall be entitled to vote at a General Meeting or to receive the Association's publications, nor shall any publication be sent to a new member until his entrance fee and subscription shall have been received.

The Council may remove from the roll of the Association any member whose subscription is one year or more in arrears.

VIII. Honorary Members shall be persons, not subjects of the British Crown, who have contributed to an eminent degree to the advancement of the Science of Applied Biology. They shall be recommended by a majority of the whole Council and elected in the same manner as Ordinary Members. The number of Honorary Members shall not exceed twelve and not more than two shall be elected in any one year.

Honorary Members shall each receive a copy of the Association's publications, but shall not be liable for the payment of an entrance fee or annual subscription.

Their privileges shall be the same as those of Ordinary Members except that they shall not be entitled to vote at any election or meeting of the Association.

IX. The business of the Association shall be conducted by a Council consisting of a President, a Treasurer, the Secretaries, of whom there shall be two; one representing the Botanical, the other the Zoological Sections of the Association, the Editors of the Journal, of whom there shall be two, and twelve Ordinary Members. Two members of the Council shall be nominated by the President to act as Vice-Presidents.

X. All properties of the Association, both present and future, shall be deemed to be vested in the Council of the Association for the time being, in conformity with the provisions of the Literary and Scientific Institutions Act, 1854.

XI. The Council shall meet at such times as they may determine; six members shall form a quorum.

XII. The Council shall have the power to fill any vacancies among its number that may occur other than those resulting from the selection for annual retirement from the Council referred to in Law XVII.

XIII. The Council shall have power, at any of their meetings, by two-thirds of the votes of those present and voting, to recommend the removal from the roll of membership of the name of any member for the reason that in their opinion it is contrary to the interests of the Association that he shall remain a member. Such recommendation shall be submitted to the Association at the next General Meeting. For the ejection from the Association of any member two-thirds of the votes of the members present and voting shall be cast in favour of such ejection.

XIV. The Council shall appoint a Publication Committee consisting of the Editors, the Treasurer, two Ordinary Members of the Council, and two Ordinary Members of the Association, who shall be responsible for the publication of the Journal of the Association.

XV. The Council, at a meeting prior to the Annual General Meeting, shall appoint one or more Auditors to audit the Treasurer's accounts.

XVI. The Council shall purchase such books, instruments, specimens, furniture and other necessaries as may be required, pass the accounts and authorise their payment, and generally manage the affairs and administer the funds of the Association.

XVII. At a meeting prior to the Annual General Meeting the Council shall elect fourteen members of the existing Council and four members of the Association, not members of the existing Council, whom they recommend to the Association for election into the Council for the ensuing year. Any member of the Council vacating office shall not be eligible for re-appointment as an ordinary member of Council until after the lapse of twelve months. The list as drawn up by the Council shall be sent to all members resident in Great Britain and Ireland at least four weeks before the date of the Annual General Meeting. It shall be competent for any member, on receipt of the recommendations of the Council, to add the name of a member or members of the Association to the list of candidates for election to the Council; such additional nominations, duly seconded, must be in the hands of one of the Secretaries not less than fourteen days before the Annual General Meeting.

The Secretaries shall when necessary, and not less than seven days before the Annual General Meeting, issue to every member of the Association resident in Great Britain and Ireland a completed list of the proposed Officers and Council for the year, indicating the names of the proposers and seconders of candidates other than the Council's nominees.

The election of new Officers and Council shall be conducted in the following manner:—At the Annual General Meeting each member present shall receive the list of Officers and Council proposed for the year. If no additional nominations have been received after the Council's recommendations, the list shall be put to the meeting and voted on by a show of hands and the result declared by the Chairman. If additional nominations have been received a ballot shall be taken; each member voting shall hand in person to one of the Secretaries a copy of the list on which has been indicated the names of those candidates whom the member voting desires to serve on the Council. When the ballot has been declared closed the Chairman shall appoint from among the members present, two persons, not candidates for election, to serve as Scrutineers. In examining the lists handed in the Scrutineers shall set aside and take no account of any ballot paper which supports candidates for more than the number provided for in Law IX, nor of any ballot paper which indicates the identity of the member voting. The Scrutineers shall report to the Chairman of the meeting the result of their scrutiny, and the Chairman before the close of the meeting, shall announce the result of the ballot. In the case of an equality of votes for any candidates, the power of selection between them shall rest with the Chairman of the meeting and shall be exercised before announcing the result of the ballot.

XVIII. The Association shall meet at times and places to be decided by the Council.

At all Ordinary General Meetings ten shall form a quorum (see also Law XIX). All meetings shall be announced by circular addressed to each member resident in Great Britain and Ireland. At all Ordinary General Meetings the order of business shall be decided by the Chairman.

An Annual General Meeting shall unless otherwise decided by the Council be held at the date of the Ordinary General Meeting falling nearest to the beginning of the year.

At this General Meeting the order of business shall be:—

1. The reading of the minutes of the previous meeting.
2. The reading of a report of the Council on the work of the past year.
3. The statement of the Treasurer.
4. The election of Members.
5. The election of Officers and other Members of the Council.
6. Other business.

A Special General Meeting may be called to discuss or take action upon any matter affecting the interests of the Association.

A Special General Meeting shall be called either by the decision of the Council or at the request of at least ten members addressed to the Secretaries.

XIX. No new law shall be passed nor any standing law altered or added to, nor any other change in the constitution of the Association made except by a Special

General Meeting of which for this purpose a fourteen days' notice must be sent to all Members resident in Great Britain and Ireland.

The requisition for such a Special General Meeting duly signed and stating in writing the laws proposed or the alteration desired, must be delivered to one of the Secretaries, who shall within a reasonable period, call such a meeting. The proposed new laws or alterations in the laws shall be printed in the circular convening the meeting.

At a Special General Meeting convened for the purpose of altering the constitution or amending the laws, fifteen shall form a quorum and no motion can be passed except by a two-thirds majority of those present and voting.